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YELLOW FEVER VACCINATION

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INTRODUCTION

First applied in French West Africa in 1934 and then in Brazil in 1937 systematic vaccination against yellow fever has since resulted in the immunization of tens of millions of persons and human yellow fever has become a rare disease When vaccination was begun however not even the most optimistic of its advocates could have anticipated the benefits of all kinds and the enormous economic and social advantages that would accrue to those territories where the disease was rife

The World Health Organization considered that it would be appropriate at this stage in the development of yellow fever vaccination to publish a monograph on the subject to which would contribute eminent specialists whose names are linked with the success of yellow fever control the world over

The first of these contributions is that of K C Smithburn in which he describes the basis on which yellow fever immunization now rests

In French West Africa and French Equatorial Africa more than 56 million vaccinations were performed between 1939 and 1953 with the scratch vaccine prepared by the Institut Pasteur at Dakar these have resulted in a protection rate such that human yellow fever has practically disappeared from the territories in which vaccination was performed C Durieux formerly Director of the Institut Pasteur at Dakar describes the various stages in the preparation and control of the vaccine produced by that Institute the technique used for its administration and the system of mass vaccination applied in French African territories Together with R Koerber he also discusses the immunological results obtained

The most widely used vaccine in the rest of the world is 17D vaccine The Oswaldo Cruz Institute at Rio de Janeiro is one of the most important establishments preparing it H A Penna who directs the Institute's yellow fever laboratory gives details of the methods of production and administration of 17D vaccine This vaccine like that of the Institut Pasteur at Dakar is used in mass vaccination campaigns C de Souza Manso reports on what has been accomplished with these campaigns in Latin America

G Courtois reports the results of numerous control experiments designed to clarify previous knowledge of the time of appearance of immunity to yellow fever after vaccination and of the duration of that immunity

Like all vaccination that against yellow fever has sometimes been followed by more or less serious reactions It is necessary to know about these accidents and only proper to draw attention to them they in no way detract from the value and efficacy of the method G Stuart gives a detailed and complete account of reactions observed to vaccinations with both the Dakar and the 17D vaccines

Each method of vaccination has its advantages and its disadvantages Vaccination with 17D vaccine by the scarification method described by G W A Dick however would seem to combine the advantages of both Dakar and 17D vaccines without the possible disadvantages of the more usual practices of administration of either

Vaccination against yellow fever is officially sanctioned as a preventive method by international health legislation which allows travellers holding a valid certificate of vaccination to avoid quarantine isolation when passing from an area infected with yellow fever to an area receptive to it P H Bonnel describes the international regulations governing yellow fever vaccinations and the conditions under which a valid international certificate may be issued

The monograph also contains a bibliography of works on yellow fever immunology and vaccination

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IMMUNOLOGY OF YELLOW FEVER

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Yellow fever was the first disease in man shown to be caused by an ultra microscopic filtrable virus [8, 91] The workers who made this dis covery were further successful in establishing yellow fever as the first virus diesease *in man known to be transmitted by mosquitos These epoch* making discoveries had far reaching effects in the control of the disease but further advancement of knowledge concerning it was delayed another quarter century until a susceptible animal host was found [84] The great strides made in research on this disease since 1927 have gone far in bring ing it under control They have also served as models for work on other insect borne diseases

A comprehensive review of the more significant research on yellow fever was published under the editorship of Strode in 1951 [85] The chapter on immunology in that volume covered the subject more extensively than is the purpose of this publication and included much material not pre viously published In this communication the subject will be treated especially in its relationship to vaccination and the more significant original papers appearing since 1951 will be considered

Immunity to yellow fever may be either active or passive Active immunity to the virus may result from natural infection from experimental infection or from vaccination Passive immunity may be the result of transfer of immunity from an immune mother to her offspring or it may result from the inoculation of an otherwise susceptible individual with protective antibody derived from an immune individual

Active Immunity

Active immunity resulting from natural infection

Even before a susceptible host for yellow fever virus was known it was recognized that a human being who contracted yellow fever and recovered became immune as a result of this experience and did not con *tract the disease a second time in his life* [54, 82, 94] *Soon after the rhesus*

monkey was found to be susceptible to yellow fever virus [64] it was observed that the serum of persons recovered from the disease was effective in preventing infection in monkeys [64] when given along with or at about the time of the inoculation with the virus Advantage was taken of this fact particularly in the laboratories of the Yellow Fever Research Institute at Yaba Nigeria to study the distribution and frequency of yellow fever in various West African countries [5 35] It was also observed that protection tests with sera from persons in the acute phase of yellow fever and with convalescent sera from suspect cases could be used for diagnostic purposes The protection test revealed also that the yellow fever of the African continent and that of South America are the same disease [31 36] These uses of immunological methods developed in 1927 and 1928 have now been superseded by a method which is more convenient largely by the fact that it is less cumbersome and less expensive—namely the performance of the same type of test in Swiss mice Nevertheless the early experiments with monkeys revealed the scope of immunological methods in diagnostic and epidemiological studies

Very little is known about the mechanism responsible for immunity to yellow fever either in man or in the animals which are susceptible to the disease It is known that the development of immunity is associated with the appearance in the serum of antibody which is capable of neutralizing the virus but it is not known whether other defensive mechanisms than the humoral are involved The inoculation of virus into an immune host does not elicit a visible reaction even though the inoculation be made intradermally in a site where even a mild inflammatory reaction would be quite easily detected Nevertheless the virus is quite effectively dealt with in the immune host and can rarely be recovered once it has been injected

The antibody response evoked in man by natural infection with yellow fever virus occurs fairly rapidly [6] and even in fatal cases in which death occurs as early as the fourth or fifth day the antibody can be detected as a rule before death Once this response has taken place in a human being the individual apparently never loses his immunity Neutralizing antibody has been demonstrated as long as 75 years after an attack of the disease [64] Further evidence of the enduring nature of the immune response is the fact that authenticated instances of plural infection in the same individual with yellow fever virus are unknown

Certain wild animals which are natural hosts of yellow fever in either Africa or South America in particular the wild primates exhibit an antibody response to the infection which is in all respects similar to that characteristic of the human being The duration of the response in monkeys is prolonged as it is in man This enduring character of the response both in the human being and in the wild animal (monkey) host is taken

advantage of in epidemiological studies to determine the presence and density of infection occurring under natural conditions [2, 21, 29, 40, 41]

Active immunity following experimental infection

All the characteristics of the response which experience has shown to be typical in human beings infected with yellow fever can be detected in experimentally inoculated primates If a susceptible monkey is inoculated with a small dose of yellow fever virus or is subjected to the bite of one or more infected mosquitos of a vector species a typical infection results [84] If the animal is of a highly susceptible species such as the Indian rhesus monkey (*Macaca mulatta*) and especially if the infecting virus is an African strain death is almost certain to result but if the animal is bled when it is *in extremis* and the serum is inactivated to destroy any virus which may be present neutralizing antibody is almost certain to be detectable In the event of the survival of the animal antibody is usually detectable three or four days after virus was first demonstrable in the serum In experimentally inoculated primates as in human beings and in monkeys naturally infected the antibody once it has appeared in the serum is likely to be present for the remainder of the life of the animal A monkey which has exhibited such an antibody response is completely refractory to re inoculation with yellow fever virus regardless of how susceptible it may initially have been

Both the human being naturally infected [8] and the monkey experimentally inoculated with yellow fever virus thus exhibit the stage of the disease in which both the virus and the specific antibody are demonstrable in the blood At this stage of the disease the virus is of course diminishing in quantity and the antibody is on the increase This phase of the infection in monkeys occurs over a 24 48 hour period between the third and fifth or sixth days after inoculation with any but the very smallest doses of the virus With minimal doses of virus the entire clinical reaction may be delayed as was shown by Bauer in experiments in which yellow fever virus was titrated in rhesus monkeys [2] In those experiments animals receiving minimal infective doses exhibited no response over a long period of time but when the infection began to take place it was typical in all respects including the antibody response of infection with a shorter incubation period

Active immunity induced by vaccination

The practical aspects of this question are dealt with by Courtois in his contribution to this monograph (see page 105) and we shall concern ourselves here with historical and theoretical aspects of the matter

The first vaccine against yellow fever was developed by Sawyer Kitchen & Lloyd[66] The method they proposed consisted in the inoculation of a measured dose of neuro adapted yellow fever virus together with sufficient specific immune serum to nullify any harmful effects which the virus might otherwise have had This method was tried originally in monkeys and was found to be quite effective When the method was applied to human beings the earliest recipients of the vaccine were hospitalized and quarantined and kept under very close observation Although some of them developed mild clinical reactions none of these was serious The antibody response was good and the sera of vaccinated individuals showed high protective powers[67] Field application of the method was greatly restricted however owing to the prohibitive amount of specific immune serum required Convalescent human beings or vaccinated persons were the original sources of supply for immune serum but when this source became inadequate the hyperimmunization of monkeys[89] or of non susceptible hosts such as horses and goats was undertaken in order to meet the demand Even these sources would not have been adequate on the large scale to which the vaccine came to be used

Workers in various countries applied themselves to the problem of developing a vaccine for yellow fever but the first vaccine to be used widely in the field was the attenuated strain developed by Lloyd Theiler & Ricci[48] A strain 17E[4] was first used and then shortly replaced by the 17D strain[90, 91] which was apparently more nearly fixed in its properties This strain was extensively studied in the laboratory before its field application was undertaken[91] The first large experiment in the field with 17D vaccine was that of Smith Penna & Paoliello in Brazil[73, 8] These workers concerned themselves especially with the technical aspects of the problem for instance the transportation of a thermolabile product and administration of the material to significant population groups within the time which the lability of the material allowed Originally bio-assays were also carried out on each lot of vaccine and sampling of vaccinated persons was undertaken before and after the inoculation to study the antibody response It was found that demonstrable antibody was evoked in a large percentage of cases by inocula which contained 100 or more LD_{50} of the 17D vaccine With smaller doses the response of some individuals was equally good but the percentage exhibiting a response was lower Reactions to the vaccine were uncommon and mild when they did occur[73, 83] Accordingly the 17D vaccine was adopted for routine use in Brazil and other South American and Central American countries as well as in certain parts of Africa

With the outbreak of the Second World War the Rockefeller Foundation undertook to supply yellow fever vaccine 17D to the armed forces of the United States of America and allied countries[85] During the course

of the war more than 25 million doses of this vaccine were produced in the Foundation laboratories and dispensed to American and allied governmental agencies In the early years the vaccine was made with a component of non immune human serum originally believed to be necessary to protect the virus against deterioration Vaccine prepared in such a manner was responsible for causing a large number of cases of hepatitis [23] especially among American military personnel [69, 70, 93] owing to contamination with the relatively thermostable hepatitis virus contained in the human serum component Before this Theiler had already carried out experiments which showed that effective vaccine could be prepared without the use of serum When this series of hepatitis cases occurred the large scale production of vaccine prepared without serum was undertaken in the United States Public Health Service laboratories [81] and the laboratories of the Rockefeller Foundation in New York Since that time no cases of hepatitis have been reported following vaccination with the 17D strain

The antibody response to 17D vaccine in rhesus monkeys has been studied by Theiler & Smith [91] and by Smithburn & Mahaffy [8] Inoculated monkeys seldom exhibit the vaccine virus in their circulation but they respond by the formation of specific antibody which is demonstrable in the blood within about seven days following inoculation Experimental challenge however reveals that monkeys vaccinated with 17D virus are refractory to the pantropic virus within about three or four days from the time they receive the 17D vaccine Thus the animals are actually refractory to the inoculation three to four days prior to the time when antibody may be demonstrated in the blood Whether this is due to the presence of antibody in quantity too small to be detected by the available methods or whether it is due to the fact that the developing immunity becomes effective during the incubation period which the pantropic virus requires is not known

Administration of the 17D vaccine to man is followed within seven to ten days by the appearance in the serum of demonstrable specific antibody capable of neutralizing yellow fever virus [5, 81] In field application the percentage of persons responding to the vaccine is very high Occasional failures occur under field conditions but in the experience of the writer where yellow fever vaccine was administered to newly engaged employees in a field laboratory and where the responses were studied by protection tests no failures were observed over a period of several years Such failures as do occur in field experience are probably due to vaccine of inadequate potency or to faulty technique in its administration

According to Dr F L Soper production of 17D virus vaccine without a human serum component was begun and made the standard practice for all yellow fever vaccine produced in Brazil after August 1940 by Dr J A Kerr in the laboratory of the Service for Study and Research on Yellow Fever maintained by the Ministry of Health of Brazil and the Rockefeller Foundation This fact is not generally known

state with certainty that such efforts were successful in preventing infec tions but there is reason to suspect that they may have been

Demonstration of Immunity by Means of Protection Tests

The first method devised for the performance of yellow fever protection tests involved the use of rhesus monkeys [81] In the early surveys in which these animals were used to study the distribution of yellow fever in the human population of West Africa [5, 35] two rhesus monkeys were used for the testing of each individual serum Thus the method was extremely costly and its application accordingly limited However with the dis covery in 1930 by Theiler [86] that yellow fever virus is pathogenic for Swiss mice when inoculated into the brain of these animals the way was laid open to more practical methods for the performance of protection tests Theiler showed that yellow fever immune serum mixed with the virus and inoculated intracerebrally had a neutralizing action and prevented infec tion in mice [87, 88] The view was held at this time however that immunity could be more effectively demonstrated if relatively large quantities of serum were administered Sawyer & Lloyd [63] developed the first extra neural protection test technique in order to effect the administration of as much serum as possible According to their method 1 5 ml quantities of concentrated mouse brain virus suspension were added to 3 0 ml quantities of undiluted serum and the mixture was inoculated intraperitoneally in amounts of 0 6 ml per individual into mice previously prepared by the intracerebral injection of a small quantity of sterile soluble starch solution The latter agent caused slight irritation in the brain and brought about the localization of the yellow fever virus which however had already been exposed to the in vitro and in vivo action of the serum with which it was mixed If the serum contained the specific antibody the mice were pro tected thereby if it did not fatal encephalitis resulted This method was used in the International Health Division laboratories of the Rockefeller Foundation in New York [65, 1] in the laboratories of the Yellow Fever Service in South America [9, 80] at the Yellow Fever Research Institutes at Lagos [6, 7] and Entebbe [77] and elsewhere [45, 46] in the intensive world wide surveys of immunity to this disease Later when the protection test came to be used more extensively for other purposes than immunity sur veys more sensitive methods were required In various studies of the immune response evoked by vaccines against yellow fever it was found that the antibody response in man is less intense than that which follows natural infection and in order to detect the response to the vaccine with certainty more sensitive methods were required also in epidemiological

studies of the role of wild animals in natural cycles of infection it was found that some animal hosts respond to yellow fever virus less intensively than does man and likewise required the use of more sensitive methods Consequently various modifications of the yellow fever protection test were proposed in particular by Whitman[95] Bugher[9, 10] and Smithburn[4] in order to meet one or more special needs for the test In principle all these modifications have much in common and they differ from the original methods proposed by Sawyer & Lloyd[68] principally in employing a more sensitive balance between the amount of serum and the dose of virus[71] or better standardized virus preparations[9] or more sensitive experimental hosts[10, 4, 95] It was found that a method which was satisfactory in one laboratory[95] might not necessarily be equally satisfactory in another[71] owing to differences in the susceptibility of the mice used in the different laboratories Likewise some of the methods especially those involving the use of lyophilized virus preparations[9] were not applicable everywhere owing to lack of facilities for the preparation of the standard virus As a result of these facts laboratories in various parts of the world came to use not so much a standard technique for the yellow fever protection test as a technique which was found to be suitable for a given laboratory and for the particular investigations in progress there

There are a great many variables in yellow fever protection tests by whatever methods they are carried out Mice of different breeds are extremely variable in their response to this virus as was found years ago by Theiler[89] Theiler likewise observed that infant mice are much more susceptible to yellow fever virus than adult mice[89] these variations in susceptibility were more critically explored in later years by Whitman[95] and Smithburn[4] Another important variable in the technique of the test is the route by which the animals are inoculated In adult mice the virus *must be introduced intracerebrally or if it is inoculated extraneurally* an intracerebral inoculation of a localizing agent must be given Even with the use of intracerebral injections of starch solution however peripheral inoculation of virus is much less effective and multiple intracerebral infective doses are required to cause illness[4]

The dose of virus used in the yellow fever protection test is of the utmost importance By whatever route the virus is introduced it is possible to overcome the effectiveness of antibody by excessive virus dosage[4] The *effective* virus dosage is not invariably the same as the *number of* infective units administered multiple intracerebral infective doses are required to produce illness if the virus is introduced peripherally—even with simultaneous brain injections of starch solution Also by *one route* of inoculation the antibody may be more effective against the virus than by another route higher antibody titres are obtained with the same serum in intraperitoneal tests in immature mice than in intracerebral tests[4]

When protection test are being done for the purpose of identifying *newly isolated strains of (pantropic) yellow fever virus* the intracerebral route of inoculation is the one of choice since freshly isolated virus may be of low virulence on peripheral inoculation—even with preparatory intracerebral injection of starch solution (Strode [85] pp 226 227)

The strain of virus being used in the tests may also determine certain technical features For example J H S Gear B de Meillon and D H S Davis [b] used the 17D virus in protection test surveys in order to avoid importation of the more potent neurotropic virus into South Africa The tests done with 17D virus yield results comparable to those done with standard neurotropic virus but require that the mice be observed for a longer period owing to the slower progress on infection with this variant

False positive results are occasionally obtained in yellow fever protection tests especially with certain animal sera [39 49] Recognition of this fact and the adoption of more severe criteria for testing such sera [9] are essential if confusion is to be avoided in epidemiological studies

The quality of the serum which is being tested also has an influence on the outcome of the protection tests The writer has repeatedly titrated yellow fever virus simultaneously in the sera of different species and found the titre to be significantly higher in one than in another Bugher [9] has also pointed to the necessity of obtaining sound results with non immune individuals of any species of which the sera are being extensively studied by means of protection tests He emphasizes that the sera of one species may require to be tested by a somewhat different method (virus dosage) from that used for routine purposes Furthermore the sera of certain animal species have been found to have toxic properties for Swiss mice and to require special methods In our own experience the serum of the ratel or honey badger (*Mellivora capensis* Schreber) behaved in this manner and could not be tested in the undiluted state Dilution to a concentration of 1 10 eliminated the toxic action sufficiently to permit performance of tests with sera of this species

The distribution of virus in the suspension used is very important and the results will be poor whatever method is used if uniform dispersion is not obtained When fresh mouse brain virus is being used as in the case of intraperitoneal mouse protection tests considerable care and patience must be exercised in the preparation of the suspension In instances in which preserved virus (frozen or lyophilized material) is used the suspension prepared for stock must likewise be made carefully and certain precautions exercised when this material is re constituted for the performance of the tests For instance lyophilized virus which is rehydrated and

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[b] Paper presented at the African seminar on yellow fever at Kampala Uganda 8 September 1953

titrated immediately is likely to give a lower titre than if the rehydrated material is allowed to stand for 15 30 minutes before the dilutions are prepared for titration This is probably due to the fact that complete dispersion of virus particles in the liquid does not take place immediately

It is essential to employ a protein containing diluent for the virus owing to its sensitivity to electrolytes [4] The materials most commonly used for this purpose are non immune serum in 5 % or 10 % concentration [4] or bovalbumin [18] The concentration of bovine albumin recommended by Dick & Taylor [18] is however inadequate and should be increased to 0 75 %

The technique of inoculation may likewise be a source of variability in the performance of protection tests especially if these be done by the intracerebral route Individuals who are not practised in the technique of inoculation may vary their procedure sufficiently to influence significantly the outcome of the tests Furthermore even if the technician is fully experienced and practised the method which one uses may be sufficiently different from that employed by another to give significant differences in titrations (Strode [85] p 191) It is therefore essential that all inoculations in a given test be done by one person and that the method in successive tests be suited to the technique which the inoculator uses

The ideal for the performance of yellow fever protection tests or any other immunological tests would be to adopt a standard technique of procedure which would be rigidly followed in all laboratories where such tests are done In the case of the yellow fever protection test however in the opinion of the writer this is neither possible nor desirable Mice used in different laboratories vary sufficiently in their susceptibility either innately or at different ages so that a technique such as Whitman s [95] 21 day mouse intraperitoneal test might be quite suitable in one laboratory and impossible in another [4] The purposes for which the tests are being done may also necessitate variations in technique When the tests are for survey purposes and it is desired to learn whether yellow fever occurs in a given geographical area it is important that the test be made sufficiently severe to eliminate any possibility of non specific or false positive reactions it is of considerably less importance in such instances to determine precisely the percentage of persons who are immune In other instances for example in studying the results of vaccination to determine how many individuals of a group have exhibited an antibody response or in studies to determine the duration of immunity induced by vaccination it does seem desirable to employ highly sensitive methods for the protection test since the detection of minimal amounts of antibody is important Therefore it may be less important to adopt a standard technique for the protection test than it is to be certain of the competence of the individuals performing the tests and then permit variations of method according to the objectives

Other Immunological Reactions in Yellow Fever

The other immunological reactions which have been found to be useful in *yellow fever* investigations are the complement fixation test and the haemagglutination inhibition reaction A number of workers have studied the complement fixation reaction in yellow fever [11, 26, 28, 33, 44, 60, 61] and a variety of different antigens some of them highly infectious have been used in the complement fixation tests It is clearly established that an attack of yellow fever gives rise to the formation of complement fixing antibody and it is likewise clear that this antibody disappears from the serum of the *infected individual usually within about 6 months* [60] *It has* also been clearly established that inoculation with 17D vaccine does not *evoke a response of complement fixing antibody* [44] *Therefore an individual* whose serum contains complement fixing antibody against yellow fever virus has not only experienced infection with that virus in its virulent form but has been infected within very recent months The failure of the serum to give a complement fixing reaction may occur in an individual who is fully immune either as a result of having had the disease more than six months previously or by virtue of having been successfully vac cinated Thus in medical or public health work the complement fixation test could be employed for diagnostic purposes [44, 61] but would be of no *value in survey work or in studying the effects of vaccination*

Recently Casals & Brown have employed complement fixation (per sonal communication) and haemagglutination inhibition techniques [13] in studying the relationships of viruses which are known or presumed to be transmitted by insects Yellow fever virus was included among these agents The complement fixation reactions obtained by them with antigens derived from yellow fever virus showed relatively little cross reaction *Good haemagglutination was obtained at pH 7 0 after incubation* at 4°C or 22°C employing antigens derived from both pantropic and neurotropic yellow fever virus *In the haemagglutination inhibition tests yellow fever* virus fell into the group B in that its antigen cross reacted with New Guinea dengue Ilheus Japanese B Ntaya Russian Far Eastern *St Louis and* West Nile *antisera* [13] *In these tests homologous* reaction was stronger than any of the heterologous reactions and the yellow fever immune serum did not react to the same extent as other antisera It is not yet clear however whether the haemagglutination inhibition reaction would be useful in field epidemiological studies

Hughes [37] conducted a series of experiments with a view to perfecting a precipitin test for yellow fever investigations *He demonstrated the* occurrence of a precipitinogen and the formation of a precipitating anti

body but found that both these reagents were of evanescent character and the test for their presence was therefore of limited usefulness

Application of Immunological Methods

The most extensive use of immunological methods in yellow fever has been in surveys to define the distribution and the prevalence of this disease in various countries in the world These surveys covered not only the known zones of endemicity in Africa [8, 7, 5–, 71, 77] and South America [79, 80] but also the remainder of both those continents as well as North America Central America Europe and parts of Asia [6] The extent to which the yellow fever protection test was used for survey purposes outstrips that of immunological tests for most other diseases Extensive use has been made of protection tests in studying the responses to yellow fever vaccine in particular to determining the duration of that response [1, 11, 16, 17, –5] As a result of these applications of immunological methods the distribution of the disease throughout the world is quite well defined as are areas in which the disease has not occurred in the past And in regions in which the susceptible human and animal hosts exist and in which vector species of mosquitos also occur but in which the disease has not occurred epidemiologists of the present day are keeping an ever watchful eye for possible introduction of this disease

The studies on the reaction of human beings to yellow fever vaccine have shown that the response is as a rule quite effective within one week of the time of inoculation of the vaccine [11, 8] and that the immunity induced by vaccination is well sustained [1] Studies made six [17] nine [16] and twelve years after inoculation of 17D vaccine have shown a high percentage of the recipients to have demonstrable immunity Since the 17D vaccine is a living although attenuated virus it is possible that the immunity which it induces will be found to be lifelong as is that induced by an attack of the disease

Immunological methods neutralization and complement fixation tests in particular have further important use in the identification of viruses [51, 53] and in studies of their interrelationships [38, 63, 5, 76] When new strains of virus are isolated it is necessary to employ a variety of methods in order to determine their identity Those which are most widely accepted for the identification of newly isolated viruses are the immunological methods Notable examples of this are the studies of Smithburn [5, 76] and Kerr [38] on the relationships of viruses isolated in recent years in Africa and South America and the work of Macnamara [50] in identifying as neurotropic

See also the contribution by Dr G Courtois page 105

yellow fever virus the agents recovered from the nervous systems of persons exhibiting acute encephalitis following vaccination with the Dakar yellow fever vaccine

Immunological methods are also important in the diagnosis of yellow fever When a virus is isolated from blood or in fatal cases from the tissues of the deceased person the method of choice for identification of the agent is the protection test employing the newly isolated agent against known immune and non immune sera

Immunological tests may also be of diagnostic value when the virus has not been isolated The testing of paired serum samples one taken early in the illness and the second drawn when convalescence is well under way may be done either by neutralization or by complement fixation tests[44] If the test shows that the illness has evoked the formation of neutralizing or complement fixing antibodies against yellow fever virus or its specific antigen the diagnosis is firmly established

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PREPARATION OF YELLOW FEVER VACCINE AT THE INSTITUT PASTEUR DAKAR

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One of the first strains of yellow fever virus whose experimental transmission to a receptive animal opened the way to the important advances made in the study of the disease was isolated at the Institut Pasteur at Dakar by Mathis Sellards & Laigret[2] during the yellow fever epidemic which broke out in Senegal in 1927 This virus had been transmitted to *Macacus rhesus* monkeys by the bites of *Aedes aegypti* fed 24 and 31 days earlier on a patient suffering from yellow fever Early in 1928 the virulent organs from one of the monkeys infected in this way were transported in a frozen state to Europe and America where they were placed at the disposal of various laboratories under the name of French strain

Theiler[9] studying the properties of this strain showed in 1930 that the white mouse was susceptible to intracerebral inoculation which induced a yellow fever encephalitis capable of being transmitted in series After a certain number of passages in this animal the properties of the yellow fever virus became modified so that when inoculated subcutaneously it no longer caused the death of the monkey but brought about a mild or asymptomatic illness followed by lasting immunity

The idea then arose of using this modified yellow fever virus for human vaccination In 1931 Sawyer Kitchen & Lloyd carried out the first trials on human beings by simultaneous injection of a suspension of the French strain (105th to 176th mouse passage) and a certain quantity of human immune serum

The following year Sellards & Laigret[8] suggested a method involving the subcutaneous inoculation of the modified French strain alone without immune serum

As soon as he had established a technique for this method Laigret applied it for the first time in French West Africa[1] The procedure advocated consisted of three successive injections at intervals of 20 days of

The ampoules which are sealed under vacuum and kept at a temperature of −25°C are then used as required to inoculate batches of mice intended either for maintenance of the strain or for preparation of vaccine

When the reserve of virus decreases the contents of an ampoule from the oldest brain passage are made into a suspension and inoculated into some 50 mice The brains of the paralysed animals are harvested immediately frozen dried at a low temperature distributed into separate tubes sealed under vacuum, and stored at −25°C They constitute a new reserve batch

Various trials have shown that the average weight of the fresh brains is 0 40 g which falls to 0 10 g on desiccation

A 10^{-6} dilution of the virus maintained in this way regularly kills six mice out of six

Mice

The white mice used are bred in an experimental station established by the Institut Pasteur in 1944 at an isolated site 15 km from the city of Dakar Animals imported from France were first used for breeding but in 1947 the stock was entirely renewed from breeders supplied by the Yellow Fever Research Institute at Lagos Nigeria

The total number of mice which includes an average of 2 000 breeders varies from 6 000 to 8 000 The quantity supplied each year to the laboratories of the Institute varies between 18 000 and 20 000 mice the animals being used when they have reached the age of 2½ 3 months Their weight is then approximately 17 g

No variation has been found at Dakar in the behaviour of mice inoculated with the French strain Paralysis regularly appears on the fourth or fifth day and death ensues within the following 24 hours

Inoculation

On three mornings a week a batch of one hundred mice is inoculated in order to meet the demand for vaccine

Ten glass jars are prepared in advance Each of them is provided with sufficient grain to feed the animals and given a label indicating the passage number the number of the corresponding experiment record sheet and the date of inoculation

An ampoule containing one fifth of a dried brain is opened and emptied into a mortar The fragments of nervous tissue are ground up with a pestle while at the same time 4 ml of isotonic salt solution are added drop by drop

The animals are presented one after the other by an assistant who slightly moistens the skull of each mouse with a tampon soaked in iodized alcohol The inoculation is administered in the right cerebral hemisphere by means of a syringe graduated in hundredths of a millilitre each mouse receives 0 03 ml of suspension As the virus strain regularly kills six mice out of six in a dilution of 10^{-6} and as a 1 50 suspension is employed the quantity injected represents about 20 000 minimum lethal doses (MLD)

As each mouse is inoculated it is placed in a jar ten mice to each jar

Harvesting

The mice are examined daily up to the end of the fifth day after inoculation All animals dying during the observation period are rejected

The brains are harvested on the fourth and fifth days only those from living but paralysed animals being used During these two days the jars are visited morning and evening At each visit mice which are clearly paralysed are transferred to an isolated air-conditioned room where the temperature is maintained at 24°C and the relative humidity at 40%-50% (as against 80%-90% elsewhere in the laboratory)

The animals are killed with chloroform one after the other and pinned to a sheet of cork The operator is provided with a set of dissecting forceps and fine scissors for each mouse wrapped up separately and sterilized by dry heat

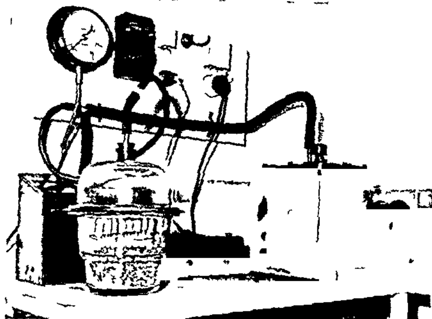
The bared skull is rendered aseptic with a few drops of iodized alcohol Once the calvaria has been removed each brain resting on the flat of the scissors is introduced into a tube plugged with cotton wool Each tube is numbered according to the order in which the mice are entered on the corresponding experiment record sheets and is immediately placed in a vacuum flask containing ice The operator then removes a fragment of the medulla oblongata with a platinum loop and seeds it in a tube of broth bearing the same number as the brain from which the sample has been taken

At the end of each session the brains harvested are stored in a refrigerator at -25 C and the tubes of seeded broth are placed in the incubator at 37 C

Desiccation

On the sixth day after inoculation all the tubes containing frozen brains from the same batch are quickly placed in a Pyrex desiccator containing Actigel (silica gel) which has first been chilled to prevent melting (fig 2

FIG 2 DRYING OF MOUSE BRAINS



The frozen b ains are pla ed in a ch lled desiccator containing silica gel The desiccator is kept at a temperature of −25°C and connected to a vacuum pump

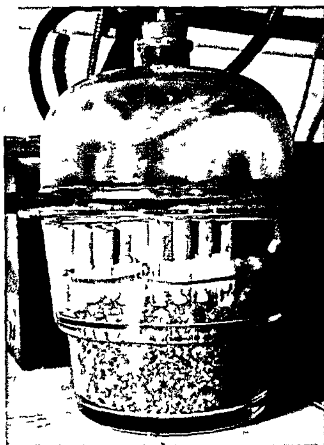
and 3) The apparatus is then placed in the refrig rator at −25°C and connected to a rotary vane pump by means of a rubber tube passing through the wall of the refrigerator The apparatus is evacuated until the Crookes dark space in a discharge tube used as a control is 20 mm long representing a pressure of 0 1 mm Hg The pump is run for several hours in the morning and evening in order to maintain minimum pressure in the desiccator

The brains are completely desiccated at the end of three days

Grinding

On the morning of the ninth day following inoculation the tubes of broth seeded on harvesting the brains are inspected and the numbers of those giving a culture noted The desiccator is then removed from the refrigerator and transferred to the air-conditioned room where it is opened The tubes containing the dried brains are then examined and those bearing a number corresponding to one on the list of contaminated broths are rejected

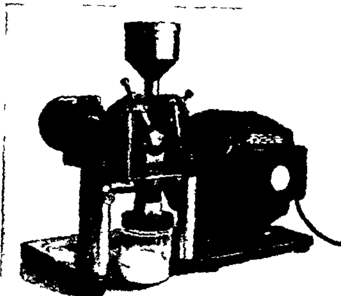
FIG 3 CLOSE UP OF DESICCATOR



All the other brains are placed together in a mortar They resemble whitish hazel nuts in appearance and are friable in consistency They are easily ground with a pestle and grinding is continued until the powder is quite uniform (In the near future this operation will be carried out by a Wiley laboratory grinder driven by an electric motor (fig 4)) A small quantity of the powder is then seeded in broth and spread on agar

Next a certain amount of Celite (purified and dehydrated infusorial earth) first filled into tubes holding 0 25 g is added to the powder One

FIG 4 WILEY APPARATUS USED FOR GRINDING DRIED BRAINS



The brains are placed in the hopper The powder obtained passes through a sieve in the lower part of the grinding chamber and falls into the glass jar

tube of Celite is required for every three brains The tubes are emptied into the mortar at regular intervals while grinding is continued so as to obtain a uniform mixture The Celite increases the volume of the powder and reduces any tendency it may have to stick to the glass

This final operation concludes the preparation of the vaccine After further seedings in broth and on agar the powder is transferred to large tubes plugged with cotton wool which are placed in a vacuum-desiccator at −25°C

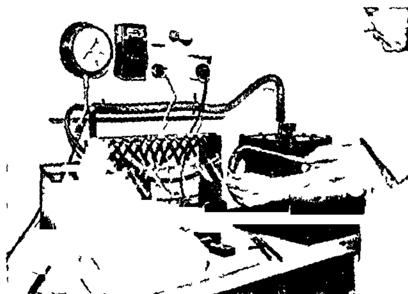
Filling into ampoules

Twenty four hours later i e on the tenth day after inoculation the vaccine is dispensed into ampoules This operation is carried out in the shelter of a Plexiglas cage installed in the air conditioned room

One measure of vaccine corresponding to one tenth of a brain is introduced into each ampoule A final check of the culture media is made before the end of dispensing and the ampoules plugged with cotton

wool are placed in a vacuum desiccator at a temperature of −25°C On the following day they are sealed with a blowlamp under vacuum (see fig 5)

FIG 5 SEALING VACCINE AMPOULES UNDER VACUUM

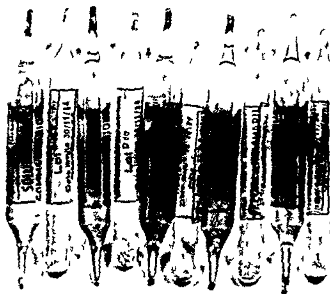


Storage and dispatch

The sealed ampoules are packed in containers bearing the batch number of the vaccine and stored at 1°C (see fig 6) At the Institut Pasteur itself they can be used for vaccination during a period of one year Ampoules to be dispatched are passed through an automatic machine which prints the batch number and the expiry date on each This date is set to ensure that the period of validity of the vaccine does not exceed two months from the day of dispatch

The vaccine can withstand air temperature for a few days without losing its immunizing properties It is sent out in ordinary parcels provided that it does not take more than about ten days to arrive or by air mail when its destination is very distant On receipt the ampoules of vaccine should again be placed in a refrigerator at a temperature below 5°C and kept there until required for use The expiry date should not be exceeded if unsatisfactory results are to be avoided

FIG 6 AMPOULES OF DRIED VACCINE AND AMPOULES OF GUM ARABIC SOLUTION



Excipient

When wanted for use the contents of an ampoule of vaccine are suspended in 2 ml of gum arabic solution prepared as follows

To 1 kg of powdered Senegal gum weighed out into a glazed dish are added 1 500 ml of tap water After stirring the solution is poured into a flask and sterilized for 30 minutes in an autoclave at 105°C

Two litres of the gum solution are measured out into a 5 litre flask In another flask 95 06 g of dry disodium phosphate are dissolved with gentle warming in 1 332 ml of distilled water

The phosphate solution is poured into the gum solution and the two are mixed sterilized for 20 minutes at 110°C and then filtered hot through Chardin filter paper

After cooling the pH is adjusted to 7 2 7 4 with sodium carbonate solution The solution is filled into 2 ml ampoules under vacuum in a bell jar and sterilized by tyndallization

Vaccine *Sterility* and Potency Tests

As indicated above the vaccine undergoes four tests for sterility in the course of its preparation

(1) test of each brain removed

(2) test of the powdered brain

(3) test of the brain Celite powdered mixture

(4) test of the vaccine at the time of filling into ampoules

Each operation is undertaken only if the preceding test has shown bacterial contamination to be absent

Occasionally tests are performed to make sure that the virus strain is pure For this purpose 1 ml of a 1 10 suspension of virus is injected intraperitoneally into each of two guinea pigs The animals are kept under observation for 15 days during which they should not show any appreciable clinical symptoms

A few ampoules from each batch of vaccine are kept in reserve at the Institut Pasteur for titration and for any tests which may be required The ampoules intended for titration are kept in the dark at laboratory temperature (an average of 28°C) the others are kept at 4 C

Titration takes place at the beginning of each month at which time three different batches of vaccine prepared at intervals of a month are examined as follows

(1) a batch two months old and exposed since then to laboratory temperature (this batch will have already undergone an initial titration immediately after filling into ampoules and a second titration a month later)

(2) a batch one month old already titrated immediately after filling into ampoules and kept since then at laboratory temperature

(3) a batch which has just been prepared

One ampoule from each batch is taken for titration The ampoule from the first batch is emptied into a mortar and the vaccine is mixed by stirring with a pestle with 4 ml of physiological saline containing 10 % of normal serum This amount of vaccine corresponds to one tenth of a brain i e 0 04 g of fresh tissue the dilution of the suspension obtained is consequently 1 100

From this suspension 10^{-3} 10^{-4} 10^{-5} and 10^{-6} dilutions are prepared in physiological saline containing 10 % of normal serum These dilutions are placed in a refrigerator at 4°C until required for the inoculations The same operations are then repeated with the ampoule of vaccine from each of the other two batches

When all the dilutions are ready the inoculations are carried out 0 03 ml of each of the 10^{-4} 10^{-5} and 10^{-6} dilutions being administered intracerebrally to six mice Titration of each batch of vaccine is thus done on 18 mice in three jars of six each The mice are kept under observation for 15 days

As a general rule the 10^{-6} dilution of the freshly prepared vaccine kills six mice out of six Consequently, 0 03 ml of this dilution contains at least one minimum lethal dose (MLD) i e 33 MLD per ml

At the time of vaccination one ampoule of vaccine (representing 0 04 g of fresh brain) is made into a suspension with 2 ml of gum arabic solution giving a 1 50 dilution If this vaccine kills six mice out of six in a 10^{-6} dilution *the vaccinal suspension will contain 660 000 MLD* per ml As the 2 ml of suspension serve to vaccinate 100 persons each separate dose of vaccine contains at least 13 200 MLD

Appreciably the same results are obtained by using the method of Reed & Muench [6] The 2 ml of suspension prepared with one ampoule of vaccine are considered in this case as the unit They are diluted with 18 ml of physiological saline containing 10% of normal serum Starting with this 1 10 dilution the successive tenfold dilutions are prepared with 10% of normal serum up to 10^{-6} and six mice are inoculated intracerebrally with 0 03 ml of each dilution The results show that the 50% end point usually lies between 1 20 000 and 1 50 000 corresponding to a titre of 660 000 to 1 650 000 MLD per ml of vaccine suspension Since 2 ml serve to vaccinate 100 persons each separate dose contains 13 200 to 33 000 LD_{50}

Titrations carried out on ampoules kept at 4°C give similar results After a year no appreciable fall in potency is observed the vaccine always containing at least 13 200 MLD per dose of vaccine

On the other hand tests carried out with batches kept at 28°C indicate a small but gradual decrease in the potency of the vaccine This decrease is not appreciable during the first three weeks whereas at the end of a month the 50% end point may fall to 1 10 000 the titre of the vaccine is then 330 000 MLD or 6 600 LD_{50} per dose The fall in activity subsequently increases and at the end of the second month of exposure to a temperature of 28°C the titre of vaccine may be as low as 16 500 MLD each dose will then contain only 330 LD_{50}

The World Health Organization has stated that for the satisfactory immunization of man each dose of 17D vaccine should contain not less than 500 LD_{50} This dose however *is injected subcutaneously* and thus introduced in its entirety into the organism As the Dakar vaccine is administered by scarification only part of the dose is absorbed Consequently it is essential for this dose to be very much richer in virus In

our opinion vaccine administered by scarification should have a titre of at least 250 000 MLD per ml and the individual dose should contain a minimum of 5 000 LD_{50}

Strict observation of the conditions indicated earlier for the transport and storage of the vaccine will ensure that at the time of vaccination the dose administered to each individual is sufficient for effective immunization

From 1939 until 31 March 1954 the Institut Pasteur Dakar had supplied over 84 million doses of yellow fever vaccine

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VACCINATION TECHNIQUE WITH YELLOW FEVER VACCINE OF THE INSTITUT PASTEUR DAKAR

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The yellow fever vaccine of the Institut Pasteur Dakar is in the form of a powder distributed in tubes sealed under vacuum Each tube bears the number of the batch of vaccine as well as the expiry date for its use and contains an amount sufficient to vaccinate about one hundred persons A 2 ml ampoule of gum arabic solution is sent out with each ampoule of vaccine

Every consignment is accompanied by a notice giving the necessary details for use of the vaccine i e concerning storage preparation of the vaccine suspension method of vaccination reactions and contra indications

Preparation of Vaccine Suspension

The following equipment is used at the Institut Pasteur Dakar for preparing the vaccine suspension test tubes 15 10 mm glass rods 6 × 150 mm watch glasses 70 mm in diameter Petri dishes 100 mm in diameter vaccinostyles All these items are first sterilized

At the time of vaccination an ampoule of vaccine is removed from the refrigerator and a file is drawn across the upper part of the ampoule which is then broken off The contents are emptied into a small test tube by inverting the ampoule and gently tapping the bottom

The two points at either extremity of an ampoule of gum solution are broken off and while the operator blocks one end with a finger a few drops of solution are allowed to fall on the vaccine while the mixture is stirred with a glass rod so as to obtain a uniform paste The remainder of the solution is then added drop by drop the mixture being stirred constantly The suspension which should be free from lumps is then ready for use

It is transferred to a watch glass which is placed in a Petri dish to protect it from dust The suspension should be used within one hour of its preparation

Method of Inoculation

A vaccinostyle is taken with sterile forceps and mounted on a pen holder It is then charged by immersing it horizontally in the vaccine suspension Three drops of suspension are placed a few centimetres apart on the outer surface of the upper arm

Three scarifications 8 10 mm in length are made through each of the drops making certain that very slight bleeding occurs The vaccine suspension is then carefully mixed with the slightly bloody serous fluid and allowed to dry for five minutes

The gum arabic solution has the advantage of being less fluid than glycerol It does not flow and solidifies fairly rapidly In a few moments it forms a fine film which covers the scarifications and thus keeps the vaccine in place (fig 1)

Mixed Vaccination against Yellow Fever and Smallpox

Inoculation by scarification makes it possible to combine yellow fever vaccination with smallpox vaccination This mixed vaccination by means of which simultaneous immunization against both diseases can be carried out with the minimum of equipment is particularly indicated when a large number of subjects is involved

For this purpose it is preferable to employ a dried smallpox vaccine able to resist the action of the air temperature in hot countries

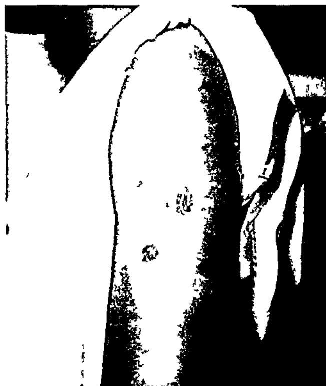
All that is necessary is to empty the contents of a 100-dose ampoule of each vaccine into a small test tube and then to grind them dry with a glass rod mixing them well The gum arabic solution is then added drop by drop so as to obtain a uniform suspension Inoculation is carried out as indicated above for yellow fever vaccine alone

Vaccination Reactions

Yellow fever vaccination alone does not cause any local reaction After a few days the scarifications heal up and disappear completely

After mixed vaccination the local reaction is similar to that caused by smallpox vaccination alone Pustules develop within the same period of time and follow the same course in both cases

FIG 1 FILM OF GUM ARABIC COVERING SCARIFICATIONS



A general reaction may appear in a certain number of vaccinated subjects in such cases it regularly develops on the fourth or fifth day after inoculation The reaction takes the form of fever headache and stiffness It usually lasts only 12 24 hours but may continue for several days with varying intensity Although rare among young persons in good health the general reaction becomes more frequent with age and the gradual wearing out of the organism and may then affect 30 % of vaccinated persons Negroes appear to be less susceptible than white people among whom women react less frequently than men

Experience has shown that after inoculation by scarification the yellow fever virus multiplies rapidly in the blood of the person vaccinated w it can be detected on the third to the sixth day It this that the general reaction appears The presence of can strated in about 60 70 % of inoculated persons by

POST VACCINATION IMMUNITY WITH YELLOW FEVER VACCINE OF THE INSTITUT PASTEUR DAKAR

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In 1931 Theiler[9] showed that the antibodies present in the serum of persons immunized against yellow fever have a protective effect when a yellow fever virus suspension mixed with this serum is injected intracerebrally into the white mouse The adaptation of this biological test to an animal in routine use made it possible to develop immunological research on yellow fever to a very considerable extent

Two procedures have so far been very widely used for detecting the presence of yellow fever antibody—the intraperitoneal method of Sawyer & Lloyd[4] and Theiler s[10] intracerebral method The second of these two has the advantage of requiring only small amounts of serum

The mouse protection test was used as early as 1932 at the Institut Pasteur at Dakar where the original technique consisted of adding the serum to be tested to a 1 100 suspension of neurotropic virus and inoculating the mixture intracerebrally into six mice This technique has since undergone various modifications in order to suit it to local conditions and to make the test more sensitive and has been very widely used as modified in surveys of endemic yellow fever in French West Africa and as a check on yellow fever vaccination

Neutralization Test Method at the Institut Pasteur Dakar

The neutralization test method employed at the Institut Pasteur Dakar consists of mixing the serum to be tested with varying dilutions of yellow fever virus and inoculating batches of white mice with these mixtures

Virus

The French strain of virus adapted to the white mouse is used A batch of 40 mice is inoculated intracerebrally with a 1 50 suspension of virus from the 258th or 259th mouse passage The brains of animals paralysed on the fourth and fifth days are harvested immediately frozen and dried in vacuo at a temperature of −25°C They are then mixed and pulverized in the mortar The powder thus obtained is dispensed into ampoules 0 10 g being transferred to each ampoule this weight corresponds approximately to that of one dried brain The ampoules are sealed under vacuum and stored at −25°C

Mice

The mice come from breeding colonies which the Institut Pasteur Dakar reorganized in 1947 from breeders sent from the Yellow Fever Research Institute at Lagos The animals used for the tests are 2½ 3 months old and weigh an average of 17 18 g

Before carrying out the test the number of mice necessary is collected (9 for each serum under test and 24 for the controls and titration) and one third of them is marked on the back with a red spot one third with a blue spot and one third with a yellow spot

Preparation of serum virus mixtures

An ampoule containing 0 10 g of powdered brain is emptied into a mortar The powder is ground with a pestle while 2 ml of 10% normal serum in saline are added with a dropper The suspension thus obtained is a 1 5 dilution since the amount of virus added corresponds to 0 40 g of fresh brain

This suspension is centrifuged for 10 minutes at 3 000 revolutions per minute after which 0 5 ml of the supernatant liquid is removed and added to 4 5 ml of 10% normal serum so as to give a 1 50 dilution From this 1 50 dilution are prepared the requisite quantities of the following dilutions 1 500 1 5 000 and 1 50 000 dilutions for the tests and 1 1 000 000 and 1 2 000 000 dilutions for titration of the virus All these dilutions are made with 10% normal serum

A quantity of 0 5 ml of each dilution is placed in haemolysis tubes arranged in racks holding three rows of 25 tubes each The 1 50 000 dilution is placed in the back row the 1 5 000 dilution in the middle row and the 1 500 dilution in the front row

The sera under test are then added Each transverse line of three tubes corresponds to one serum and bears a serial number With a pipette 1 5 ml of the first serum are taken up and 0 5 ml is added to each tube

of line No 1 beginning with the back tube (containing the highest virus dilution) The other sera are then dispensed in the same way into the three tubes of each successive line always proceeding from the back to the front row

When the quantity of serum to be tested is less than 1 5 ml 0 25 ml of each virus dilution is placed in each of the three tubes to each of which 0 25 ml of serum is then added

The six tubes of the last two lines are reserved for the control sera To each of the three penultimate tubes is added 0 5 ml of immune serum from a donkey hyperimmunized by massive inoculations of virulent mouse brain while 0 5 ml of normal monkey serum is added to each of the last three tubes

Once the mixtures have been completed all the tubes are shaken separately by hand so as to ensure contact of the virus with the sera and left for half an hour at laboratory temperature (25 30 C)

Inoculations

The serum virus mixture in each tube is injected intracerebrally into three mice 0 03 ml being administered to each animal The same syringe is used for the three mixtures of each serum The mixture from the back tube (highest virus dilution) is inoculated into three mice marked with yellow spots the mixture from the middle tube into three mice with blue spots and the mixture from the front tube (lowest virus dilution) into three mice with red spots The nine mice inoculated with the three mixtures of each serum are placed together in one jar marked with the number of the experiment the registration number of the serum and the date of inoculation

The procedure is repeated with the control sera (normal and immune) which are the last to be injected

When these operations are finished the check on the virus is completed by inoculating three mice marked with blue spots with a 1 1 000 000 virus dilution and three mice with yellow spots with a 1 2 000 000 dilution These six animals are placed in the same jar

Observation of inoculated animals

The inoculated mice are examined every morning for 10 days The results of the examination of each jar are entered daily on a printed form of eleven columns for the days of observation and nine horizontal rows numbered for the nine mice (see fig 1 and 2)

The first mouse to fall ill or die in each of the three groups is assigned No 1 for those with red spots No 4 for those with blue spots and No 7

FIG. 1. RECORD SHEET FOR MOUSE PROTECTION TEST SHOWING GOOD PROTECTION

		0	1	2	3	4	5	6	7	8	9	10
Red	1						P	+				
	2							P	+			
	3							P	P	+		
Blue	4											L
	5											L
	6											L
Yellow	7											L
	8											L
	9											L
Result = ++ (good protection)												

P = paralysed   L = living   + = dead

for those with yellow spots, and so on in numerical order. Death or the onset of paralysis is entered in the row for the mouse concerned under the day of occurrence. Deaths before the fifth day are not taken into account.

Interpretation of results

The use of different virus dilutions makes it possible to assess to a certain extent the protective power of the serum being examined. The various possible results which can be obtained after a 10 day period of observation are summarized in table I.

The mice used for virus control are regularly killed by the 1 : 1 000 000 dilution and sometimes by the 1 : 2 000 000 dilution. It may therefore be taken that each of the animals has received at least 1-2 minimum lethal doses (MLD). With the addition of an equal volume of serum the 1 : 500, 1 : 5 000 and 1 : 50 000 virus dilutions used for the tests become 1 : 1 000, 1 : 10 000 and 1 : 100 000 dilutions. Consequently mice inoculated with each of these three dilutions have received 1 000-2 000, 100-200 and 10-20 minimum lethal doses per animal, respectively.

FIG 2 RECORD SHEET FOR MOUSE PROTECTION TEST
SHOWING NO PROTECTION

		0	1	2	3	4	5	6	7	8	9	10
Red	1						P	+				
	2						P	+				
	3							P+				
Blue	4							P+				
	5							P+				
	6							P+				
Y llow	7							P	+			
	8								P	+		
	9								P	+		
Result = 0 (o protecti)												

P = pa alysed + = d d

Under these conditions the neutralizing power of a serum examined may fall within one of the following categories In the case of a serum protecting against a dilution of

1/50 000	1 ml neutralizes	600-1 200 MLD
1/5 000	1 ml neutralizes	6 000-12 000 MLD
1/500	1 ml neutralizes	60 000-120 000 MLD

Interval between Vaccination and Immunity

Experience has shown that the virus contained in the Dakar vaccine multiplies rapidly in the blood of the vaccinated subject Its presence may be demonstrated in 60 70 of cases by intracerebral inoculation of the white mouse with the subject s blood The virus usually appears on the third day after vaccination persists for a few days and disappears at the latest by the seventh or eighth day It is probable that by the time the virus disappears small quantities of antibody have already begun to form in the blood

TABLE 1 DEGREE OF PROTECTIVE POWER CONFERRED BY YELLOW FEVER VACCINATION

Ratio of mice surviving to mice inoculated				Symbol	Interpretation
1 500 virus dilution	1 5 000 virus dilution	1 50 000 virus dilution	total		
3/3	3/3	3/3	9/9	+ + +	Very good protection
2/3	3/3	3/3	8/9		
1/3	3/3	3/3	7/9	+ +	Good protection
0/3	3/3	3/3	6/9		
0/3	2/3	3/3	5/9		
0/3	1/3	3/3	4/9	+	Poor protection
0/3	0/3	3/3	3/9		
0/3	0/3	2/3	2/9	?	Inconclusive
0/3	0/3	1/3	1/9		
0/3	0/3	0/3	0/9	0	No protection

If inconclusive or contradictory results are obtained the test is repeated

Certain very sensitive methods for carrying out the mouse protection test have been suggested by Bugher [1] Whitman [11] and Smithburn [7] The use of such methods makes it possible to determine with some degree of accuracy the period between vaccination against yellow fever and the appearance of antibodies The Institut Pasteur at Dakar has investigated whether this period can also be determined by the technique it has been using for many years and which has been described in detail above account being taken not only of the number of surviving mice but also of the average survival time as advocated by Bugher [1]

Two experiments were carried out for this purpose

Experiment I

Three white persons aged about 20 years Ma Vi and Bo who had recently arrived from France and had never been vaccinated against yellow fever agreed to undergo the first experiment A preliminary mouse protection test was negative in all three cases

On 2 February 1952 they were inoculated with Dakar vaccine (batch B 8) It was administered in three series of three scarifications 8 10 mm long on the external surface of the left arm There was no noticeable

reaction during the next few days and none of the subjects interrupted his normal activities

A blood sample was taken daily from each subject during the period following vaccination The samples taken on the third to eighth days were injected intracerebrally into batches of mice for detection of virus in the circulatory system and a mouse protection test was carried out with each of the sera collected from the fifth to the eighteenth day

TABLE II RESULTS OF MOUSE PROTECTION TESTS ON SERA OF 3 SUBJECTS INOCULATED WITH DAKAR VACCINE (BATCH B 8)

Time of blood sampling	Ratio of mice killed to mice inoculated (indicating presence of circulating virus)			Mouse protection tests					
				ratio of mice surviving to mice inoculated			average survival time (days)		
	Ma	Vi	Bo	Ma	Vi	Bo	Ma	Vi	Bo
Before vaccination	—	—	—	0 9	0 9	0 9	5.5	5 1	5 4
3rd day	5 6	0 6	1 6	—	—	—	—	—	—
4th	5 5	5 6	5 6	—	—	—	—	—	—
5th	5 5	6 6	4 4	0 9	0 9	0 9	5 1	5 3	4 8
6th	6 6	6 6	4 6	0 9	0 9	0 9	5 0	5 0	5 5
7th	3 6	6 6	0 6	0 9	0 9	0 9	5.3	5 0	5 5
8th	0 6	0 5	0 6	0 8	1 9	0 9	5 1	6 1	5 6
9th	—	—	—	0 9	0 9	0 9	6 0	6 1	6 2
10th	—	—	—	1 9	0 9	2 9	7.3	6.5	7.0
11th	—	—	—	3 9	0 9	1 9	7 4	6 6	7.2
12th	—	—	—	3 9	2 9	2 9	7 5	7 7	7 6
13th	—	—	—	2 9	5 9	2 9	7 6	8 6	7 4
14th	—	—	—	6 9	4 9	4 9	8 7	8 4	8 4
15th	—	—	—	4 9	3 9	6 9	8 1	8 0	9.3
16th	—	—	—	8 9	3 9	7 8	9 6	8 1	8 6
17th	—	—	—	5 9	7 9	7 9	9 0	9 3	9 4
18th	—	—	—	5 9	8 9	5 9	8 7	9 8	9 0
41st	—	—	—	6 9	8 9	6 9	9 0	9 5	8 8
6 months	—	—	—	9 9	7 9	8 9	10 0	9.2	9 8

The results of these tests are shown in table II which indicates for each serum the proportion of surviving animals and the average survival time This is calculated by the method indicated by Bugher [1] during the 10-day period of the test the number of surviving mice in each batch is noted daily these numbers are added up and the total obtained is divided by the number of mice alive on the fourth day Mice which die before the fifth day are not taken into account

Interpreting the results shown in table II according to the scale of protection indicated in table I i e taking only the surviving mice into consideration the sera of Ma can be considered positive only from the 11th day (3/9) of Vi from the 13th day (5/9) and of Bo from the 14th day (4/9) However examination of the average survival time shows that after being low between the 5th and 7th days the average survival time

begins to increase from the 9th day and then gradually rises until it approaches 10 The fall at the outset is probably related to the presence of circulating virus which adds to the effect of the virus in the dilutions used for the mouse protection test

Experiment 2

Using a sensitive mouse protection test method Smithburn & Mahaffy[8] have shown that yellow fever antibodies can be detected on the tenth day in most persons vaccinated with 17D vaccine And the Expert Panel on Yellow Fever of the World Health Organization stressed during its first session[1] the fact that there was considerable evidence to show that effective immunity was established as early as the seventh day following inoculation

The second experiment carried out by the Institut Pasteur Dakar was a comparative investigation of the appearance of yellow fever antibodies in two groups of subjects inoculated with Dakar vaccine and 17D vaccine respectively

Six white volunteers aged about 20 were used for this experiment They had recently landed at Dakar none of them had been vaccinated against yellow fever and their sera samples of which were taken before the commencement of the experiment were found to be without protective power

On 15 July 1952 three of them Ch Sa and Ca were inoculated with Dakar vaccine (batch B 66) under the same conditions as the subjects of the first experiment On the same day the three other volunteers Cl El and Hi were given a subcutaneous injection of 17D vaccine (batch 52 2) from the Institut Pasteur Paris There was no apparent reaction after these vaccinations

Blood samples were taken from the third to the nineteenth day for detection of circulating virus and then of antibody Tables III and IV show the results obtained

According to the degrees of protection shown in table I the sera tested can only be considered positive from the 10th day (3/9) and from the 11th day (4/9 and 5/9) for the subjects inoculated with Dakar vaccine and from the 11th day (5/8) the 13th day (4/8) and the 15th day (4/9) for those inoculated with 17D vaccine Nevertheless it can be seen that in both groups the average survival time begins to increase from the 7th day

The average survival time was also calculated by grouping together the sera from the six subjects of experiments 1 and 2 who had received Dakar vaccine and the sera of the three subjects inoculated with 17D vaccine For this purpose all the surviving mice in each of these two groups were counted daily The figures obtained were added up for each series

TABLE III RESULTS OF MOUSE PROTECTION TESTS OF SERA OF 3 SUBJECTS INOCULATED WITH DAKAR VACCINE (BATCH B 66)

Time of blood sampli g	Ratio of mice killed t mice inoc lated (i d c t ng prese ce of ci cu at ng i us)			Mous p ot cti n tests					
				ratio of mice surv ng to mice inoculated			a e age s rvi al tim (d ys)		
	Ch	Sa	Ca	Ch	Sa	Ca	Ch	Ss	C
Bef e vaccin tion	—	—	—	0 9	0 9	0 9	5 6	5 4	5.8
3 d day	1/4	1 4	1 4	—	—	—	—	—	—
4th	5 5	4 5	1 4	—	—	—	—	—	—
5th	5 5	2 6	1 5	—	—	—	—	—	—
6th	4 5	3 6	0 6	—	—	—	—	—	—
7th	0 6	1 5	0 6	1 9	0 9	1 9	6 4	5.8	6 4
8 h	—	—	—	1 8	3 9	2 8	6 8	7.2	7 1
9th	—	—	—	2 9	2 9	2 9	7,3	7.2	7 1
10th	—	—	—	2 9	2 9	3 9	6.8	6.8	7 4
11th	—	—	—	4 9	5 9	3 9	7.8	8 7	7 6
13th	—	—	—	3 9	3 8	3/9	7 7	8 0	8 1
15th	—	—	—	5 9	6 9	4 9	8 7	9 1	8 4
17th	—	—	—	5 9	5 9	4 9	8 7	8 6	8 4
19th	—	—	—	6 9	5 8	5 9	9.3	9 1	8 7

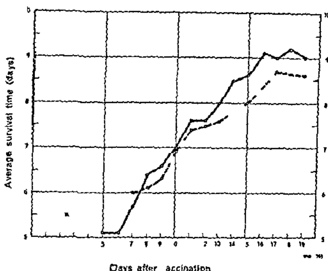
TABLE IV RESULTS OF MOUSE PROTECTION TESTS OF SERA OF 3 SUBJECTS INOCULATED WITH 17D VACCINE

Time of blood sampli g	Ratio of mice killed t mi e inoc lated (i di c t ng pre nc of ci culat ng vi us)			Mous protection tests					
				r tio of mice s r i i g to mice i o ulat d			age s vi al tim (d ys)		
	Cl	El	Hi	Cl	El	Hi	Cl	El	Hi
Befo accination	—	—	—	0 9	0 9	0 9	5 8	5 6	5 4
3 d day	0 5	0 6	0 5	—	—	—	—	—	—
4th ,,	0 4	0 5	0 4	—	—	—	—	—	—
5th	0 6	0 6	0 5	—	—	—	—	—	—
6th	0 6	0 6	0 6	—	—	—	—	—	—
7th	0 6	0 6	0 6	0 8	0 9	1 9	6 1	5 7	6 1
8 h	—	—	—	1 9	0 9	1 9	6 7	5 8	5 8
9 h	—	—	—	2 8	1 9	1 9	7 0	6 0	6 0
10th	—	—	—	2 9	2 9	2 8	6 5	6.8	6.2
11th ,,	—	—	—	5 8	2 9	2 9	8 5	6 8	7 0
13th	—	—	—	3 9	4 8	2 8	7 4	7.3	7 1
15th	—	—	—	3 9	4 9	4 9	6 3	7 7	7.8
17th	—	—	—	6 9	4 9	5 8	9 0	6.2	9 0
19th	—	—	—	4 8	5 9	4 8	8 6	8 6	8 3

and each of the two totals was divided by the total number of mice sur viving on the fourth day in the corresponding group The distribution of the averages obtained is shown in fig 3

These experiments with Dakar and 17D vaccines both confirm previous notions and show the similarity between the results obtained with the two methods of vaccination

FIG 3 AVERAGE SURVIVAL TIME OF MICE INOCULATED WITH SERA OF 9 SUBJECTS VACCINATED WITH DAKAR VACCINE OR 17D VACCINE



= a e age survival t me with the 9 serum samples taken before vaccinat on
= Dakar accine (6 sera)
= 17D vacc ne (3 se a)

The neutralization test method as employed at the Institut Pasteur Dakar thus makes it possible to get some idea of the interval between *yellow fever vaccination and the appearance of antibodies* With Dakar vaccine the presence of antibodies on the 7th to the 9th day is revealed by a progressive increase in the average survival time and then from the 10th to the 14th day by the definite protection of animals inoculated with the 1 50 000 virus suspension The protective power then gradually increases approaching the maximum towards the end of the third week

It may be affirmed that Dakar vaccine like 17D vaccine confers effective immunity before the 10th day

Efficacy of Vaccination

Having established that the French strain adapted to the white mouse was able to penetrate into the human organism through slight cutaneous scarifications and to bring about the formation of *antibodies* the Institut Pasteur Dakar undertook the first large scale immunization campaign

with this method of immunization in 1939 [4, 5] More than 100 000 inhabitants of Senegal were inoculated simultaneously against yellow fever and smallpox Only schoolchildren whose identity had been carefully established at the time of vaccination were used for control purposes Out of a total of 1 387 subjects samples of whose serum taken before inoculation had been found to be without protective power against the yellow fever virus 1 336 (96 3 %) gave a positive test 1 2 months later

The following year 35 000 Africans on the Ivory Coast and 29 000 in the French Sudan were vaccinated under the same conditions by the Institut Pasteur Dakar Controls carried out after an interval of 2 3 months also on schoolchildren gave positive results in 91 2 of cases for the Ivory Coast (400 sera tested) and 98 9 for the Sudan (198 sera tested)

In 1945 in an experiment which took place in France under the auspices of UNRRA [8] two groups of 200 white soldiers who had never left France were vaccinated by scarification Group A received only Dakar yellow fever vaccine while group B received Dakar yellow fever vaccine combined with smallpox vaccine Samples of sera taken a month later were tested in three different laboratories (Dakar Montana and Rio de Janeiro) Of the group A sera 98 9 were found to be positive (188 sera tested) and of the group B sera 97 9 (193 sera tested)

All these experiments the first two of which were carried out on large numbers of subjects under the most unfavourable conditions clearly showed that on inoculation with Dakar vaccine immunity was conferred in an average of 96% of cases

The results obtained by the Institut Pasteur at Dakar were confirmed by a test carried out in 1949 in the Republic of Panama where Courtney [2] inoculated 24 000 persons with the Dakar vaccine by scarification Of 150 sera selected at random six months after vaccination all gave a positive test

Duration of Immunity Induced by Vaccination

The Institut Pasteur at Dakar has also carried out a certain number of control tests to determine the duration of the immunity conferred by inoculation with Dakar vaccine The subjects tested were either selected at random from among the inhabitants of various territories of French West Africa or were isolated individuals

In the first case the aim was to check the immunity rate among African populations who had undergone systematic vaccination against yellow fever a few years before during tours mad by health service teams Table V shows the results obtained among the inhabitants of 14 small

villages away from main roads and lines of communication It should be made clear that samples of sera were taken without there being any absolute certainty that they all came from definitely vaccinated subjects

TABLE V RESULTS OF YELLOW FEVER IMMUNITY TESTS IN ISOLATED FRENCH AFRICAN VILLAGES

Number of years since vaccination	Terr tory	Number of sera tested	Positi e results	
			number	
2	Senegal	25	24	96 0
	Guinea	58	52	89 6
	Ivo y Coast	34	34	100 0
	Dahomey	18	11	61 1
	Upper Volta	11	7	63 6
	N ger	50	47	94 0
	Total	196	175	89.2
3	Senegal	25	25	100 0
	Suda	24	19	79 1
	Ivory Coast	40	40	100.0
	Upper Volta	25	25	100 0
	Total	114	109	95 6
4	Senegal	106	91	85 8
	Dahomey	25	22	88 0
	Total	131	113	86 2
7	Senegal	72	59	82 0

The duration of immunity was also investigated in a number of isolated persons almost all Europeans who had been vaccinated in various French or African vaccination centres All these persons were in possession of a vaccination certificate stating the date of vaccination The results of the control tests were as follows

Number of years since vaccination	*Number of persons tested*	*Number of persons imm e*	*Percentage imm ne*
1	142	134	94 3
2	89	81	91 0
3	30	8	93 3
4	17	17	100 0
5	16	16	100 0
6	10	9	90 0
7	4	3	
8	1	1	
9	7	7	87 5
10	1	0	
11	1	1	
12	2		

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17D VACCINE

PRODUCTION OF 17D YELLOW FEVER VACCINE

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Introduction

When in 1937 Theiler & Smith[11, 12] published their reports on the modification of yellow fever virus by cultivation in vitro and on the use of this modified strain (17D) for human immunization, it was evident that a safe and efficient means of vaccinating humans against yellow fever had become available.

Immediately thereafter attention was concentrated on developing methods for large-scale production and application of the newly discovered vaccine.

Most of the initial studies were undertaken in Brazil and reported by Smith, Penna & Paoliello in 1938.[9]

In the original method the virus (17D) was grown in the developing chick embryo. The infected embryos were triturated into a pulp, suspended in normal human serum, and centrifuged; the supernatant fluid was filtered through Seitz pads to ensure bacteriological sterility. The filtrate was distributed in ampoules and desiccated from the frozen state under vacuum in glass desiccators containing concentrated sulfuric acid.

Although many thousands of vaccinations were performed using vaccine manufactured by the method just described, it was found that in several ways there was room for improvement. The filtration of the material through bacteriological filters removed a large quantity of virus, resulting in many lots having to be discarded because of insufficient titre. The highly hygroscopic desiccated vaccine would absorb moisture from the air during the short time that elapsed between the opening of the desiccator and the sealing of the ampoules. The addition of normal human serum necessary to protect the virus during filtration was expensive. Also it was later found that this addition was responsible for introducing in many lots of vaccine a contamination with a pathogenic virus, then not well studied

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which is now known as the virus of homologous serum hepatitis or inoculation jaundice

As a consequence of the occurrence of a widespread epidemic of this jaundice produced by contaminated vaccines in 1939 in the State of Espirito Santo[4] all vaccinations were discontinued in Brazil and the Yellow Fever Laboratory of the Instituto Oswaldo Cruz in Rio de Janeiro began studies with a view to changing the methods of vaccine preparation Work along the same lines was being undertaken at about the same time in the laboratories of the Rockefeller Foundation in New York

The methods finally adopted in Rio de Janeiro are a combination of the techniques developed in both these laboratories and are aimed chiefly at the elimination of the serum component of the old vaccine

Since the addition of serum was necessary for the filtration of the virus the first problem was to do away with the filtration altogether and re search was directed towards devising a method of harvesting the infected embryos aseptically This was achieved by opening the eggs along a line burnt through the shell with the flame of a small blowtorch while they spin slowly in a special apparatus designed by Penna[6] and improved by Pickels[7]

For the desiccation of the vaccine the methods described by Bauer & Pickels[1] were adopted with some modifications Mechanical refrigeration replaced the original method using carbon dioxide snow for obtaining low temperatures Dry ice was not available in Rio de Janeiro at the time and it had to be made in the laboratory from cylinders of compressed CO_2

Laboratory and field experiments demonstrated that a concentrated vaccine (made without the addition of water or any other fluid and having an adequate virus content) could stand at the time of inoculation higher dilutions than the 1 10 dilution generally recommended without impair ment of its immunizing efficiency[2] This technique greatly increased the number of doses yielded per unit volume of the basic preparation and resulted in a saving in production and transportation costs materially reducing the cost per dose Although there is a certain amount of waste with vaccines prepared in this manner the advantages far outweigh the disadvantages

In 1945 the United Nations Relief and Rehabilitation Administration *according to Article XI (10) of the International Sanitary Convention for* Aerial Navigation 1944 laid down standards for the manufacture and control of yellow fever vaccine (see page 205) These will simply be referred to as the Standards throughout this paper The methods adopted in Rio de Janeiro depart in certain points from the Standards which are under review by the World Health Organization

Description of Methods

Eggs

White Leghorn eggs carefully transported to the laboratory and not more than one week old are to be preferred White shelled eggs are easier to candle Fertility should be not less than 70% This limit was established in order to save incubation space The size of the eggs should be such as not to permit their passage through a gauge having a circular aperture of 4 cm in diameter smaller eggs do not fit the rubber ring supports of the Pickels apparatus for removal of embryos

Egg shipments are received in the laboratory once a week and each lot is given a number The eggs are washed with a 1% solution of calcium hypochlorite and a soft hand brush The liquid is then drained off by setting the eggs out on trays where they are usually left overnight The number of eggs in each shipment received in the Rio de Janeiro laboratory at the present time is 2 000

Incubation

Any good commercial egg incubator will serve The temperature is kept between 37 5°C and 38 0 C The eggs are turned daily throughout the incubation period

The first candling takes place on the fifth day of incubation Infertile eggs and those containing dead embryos are discarded

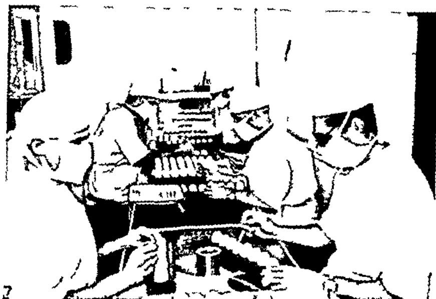
The second candling takes place on the eighth day Dead embryos are discarded and a pencil marking is made over the shadow of the living embryos The eggs are maintained with this marking face upwards until the morning of the ninth day when they are once again rapidly candled to eliminate any dead embryos The total number of living embryos is then ascertained and the lot is divided into several sub-lots of about 100 embryos each These sub lots are processed independently until the bacteriological tests indicate their sterility

Inoculation (fig 1)

The eggs to be inoculated are placed in wooden blocks each holding eight eggs in an upright position with the larger end uppermost A small area of the shell over this end is painted with tincture of merthiolate and the wooden blocks are pushed through a double door opening at table height into a dust proof room

With a very small flame from a gas oxygen torch an area not more than a few millimetres in diameter is burnt in the centre of the painted area A hole is made with a pointed instrument through the burnt shell

FIG 1 INOCULATION OF EGGS IN DUST PROOF ROOM



Wooden blocks hold ng 8 eggs each enter the room through the opening at the far end The first operator burns a small area on the shell with a blowtorch the second pierces through this area with a pointed instrument the third inoculates and the two operators in the foreg ound seal the holes Another operator not shown fills the syringes

This instrument is dipped in a beaker containing alcohol and flamed after being used on eight eggs The inoculating needle mounted on a tuberculin syringe is introduced through the hole in the direction of the embryo and 0 06 ml of the inoculum is injected One syringe is used for eight eggs and then discarded The hole is finally sealed with warm wax

Each of these operations is performed by a different person in order to ensure a continuous flow of eggs During inoculation there are six people working in the dust proof room One burning the shell one piercing it one inoculating two sealing and one filling the syringes

Tuberculin syringes of 0 5 ml capacity fitted with 1½ inch 25 gauge (38 mm × 0 50 mm) needles are used They are protected by glass tubes and packed in metal boxes for dry sterilization

Inoculum

Virus of the 17D strain originally obtained from the Rockefeller Foundation in New York should be used

The number of subcultures in embryo tissues in vitro should range between 229 and 255 [10] A moderate number of passages in the developing

chick embryo does not significantly alter the antigenicity of the virus The derivation of substrains of 17D virus used in Brazil is given by Fox & Penna[6] In the Rio de Janeiro laboratory the substrain designated EP (egg passage) has been used for several years (fig 2) This choice was

FIG 2 DERIVATION OF SUBSTRAINS OF 17D VIRUS USED IN BRAZIL

R p oduc d f m Fox & P nna slightly modif d by k nd permissio of the d to of the Am I an J al f Hyg n

based on the high immunogenicity of that substrain its lack of encephalitogenic properties and its freedom from association with the production of icterus [3] The number of passages in the developing embryo is 42 at the present time

In order to maintain the seed virus as uniform as possible the seed lot system described by Fox Kossobudzki & Fonseca[2] should be adopted It consists in preparing a primary seed lot and from it inoculating a number of secondary seed lots These secondary lots are reserved exclusively as a source of virus for the vaccine lots actually destined for

human vaccination Many secondary seed lots can be prepared from one primary lot The virus is thus maintained at a fixed passage level for a long time Seed lots are prepared in the same manner as the routine vaccine lots and stored at a temperature not higher than - 25°C

All secondary seed lots should in addition to the routine tests used on other lots be submitted to the monkey test The main purpose of this test is to guard against a possible modification of the seed virus—namely increase in neurotropism Each of six non immune rhesus mon keys should be inoculated intracerebrally with 0 5 ml of rehydrated seed lot material

All monkeys should become immune as determined by the mouse neutralization test performed on samples of blood collected 30 days after inoculation Paralysis should not occur in more than two of the animals and death due to encephalitis in not more than one

Dust proof room

All operations requiring bacteriologically aseptic techniques should be performed in a dust proof room (This room in the Rio de Janeiro labora tory measures 9 m × 3 m and the walls are covered with glazed tiles) Walls floor and tables should be washed with a solution of Lysol before the work is begun The air coming into the room should be filtered through oiled glass fibre or an equivalent filter refrigerated if necessary and preferably irradiated by ultraviolet light Slight positive air pressure should be maintained inside the room to prevent the entrance of dust when the door is occasionally opened At each end of the room there is a small hatch—with two sliding doors to allow for the passage of material into and out of the room without the formation of air currents

Operators should use sterilized gowns caps and masks A container with pieces of gauze soaked in a weak solution of iodine or similar dis infectant should be kept at hand for periodical disinfection of arms and hands during the course of operations

Post inoculation incubation

Incubation should be maintained at the same temperature as before 37 5 38 0°C Not less than 5 000 LD_{50} should be injected into each egg if the post inoculation incubation period is three days In some labora tories four days are allowed for this incubation period in that case the concentration of virus in the inoculum should be lower to avoid excessive mortality and the pre inoculation incubation period should not exceed eight days so as to keep the age of the embryos at harvesting time below 12 days

Harvesting of embryos (fig 3)

On the morning of the day when the embryos are to be recovered the eggs should be carefully candled and only living embryos retained After examination the eggs should be placed in a freezer (–20 C) where they should remain for not less than half an hour When the eggs are to be taken to the dust proof room for opening they should be removed from the freezer in the order in which they were put in

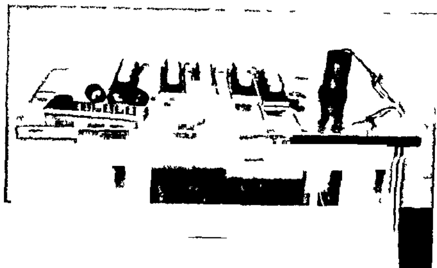
FIG 3 HARVESTING EMBRYOS



W th bl wto h th op t I th f eg o d b ns i g a o d ach gg i th P kels m ch The t man p i p n th h ll c p Th thi d ope t coll t the mb yos a d pl c th m i the t f er f th bl d which ft into pe ng mad the tabl top St ll d fo c ps e tak f m fl t m tal bo tack d I fro t f the op tor A p otect glas h eld I placed the t bl

For the removal of the embryos the technique described by Pickels[7] has been adopted in the Rio laboratory The eggs are placed with the larger end upwards in special metal trays each having four rubber ring supports which can be made to rotate by bringing them in contact with a driving mechanism (fig 4) As the eggs rotate one by one a very hot flame from an oxy acetylene torch is applied tangentially just below the margin of the air sac A ring is burnt at this level without significantly heating the interior of the egg This operation once begun is kept going continuously Another operator exposes the embryos by lifting the caps

FIG 4 PICKELS MACHINE FOR OPENING EGGS



of the egg shells with the blade of a spatula sterilized by flaming The next operator removes the embryos with sterile forceps and drops them into the metal containers of Waring blendors

All the embryos from one sub lot (originally composed of one hundred embryos) are placed in a separate container After these containers are filled they are placed in a refrigerator while awaiting the next procedure grinding

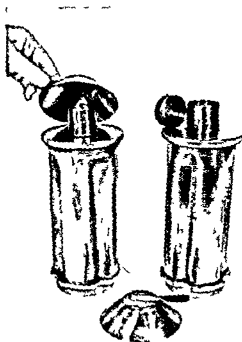
All operations connected with the harvesting of the embryos are also carried out in a dust proof room and the same aseptic precautions prevail as for inoculation A glass hood provided with an ultraviolet sterilizing lamp is mounted on the table to provide added protection in the actual opening of the egg shells and removal of the embryos

Despite these precautions contamination does occur in a certain pro portion of the embryo pulps It is probable that some contamination may be derived from the egg shells or even from the interior of the eggs Gram negative rods are the most common contaminants The percentage of contamination in the last ten lots of eggs opened in the Rio de Janeiro laboratory *at the time of writing comprising 112 sub-lots of embryos* was 10 9 The average number of embryos per sub lot was ninety

Grinding of embryos (fig 5)

Grinding is performed in Waring blendors for about six minutes However if the temperature of the material rises above room temperature grinding is interrupted for re-chilling

FIG 5 WARING BLENDOR METAL JARS FOR GRINDING EMBRYOS



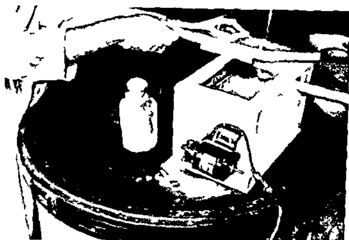
The lids of the contai e ha e been m dified t ed ce the isk of ontami at g the embryos when th y e d opp d in

Sterility tests and free ing of embryo pulps

A 1 0 ml sample from each blendor is taken for a sterility test The remainder is transferred to 1 litre Pyrex bottles which are hermetically sealed with tight fitting rubber caps Over the caps a thin sheet of rubber is stretched and held by strong rubber bands The bottles are numbered and kept cold until their contents are shell frozen This takes place in the interior of a box provided with rollers driven by an electric motor (fig 6) rotating them at a low sp ed Cold alcohol from a special freezing machine is pumped into the box and flows back into the freezer thus maintaining a continuous circulation A few minutes rotation of the bottle in such a bath (80°C) will result in the formation of a hollow cylinder of frozen pulp against the side of the bottle

The same freezer may be provided with a compartment where the bottles with the frozen pulp can be stored at 80°C If there are many flasks a separate freezer may be used especially for this purpose

FIG 6 SHELL FREEZING THE EMBRYO PULP



A small moto d es two rolle s inside the box The flask contai ing the emb yo pulp rests on the rollers and rotates slowly Cold alcohol (−80°C) f om the deep-f eeze is pumped into the box thro gh the pipe at the side and is ret ned through an outlet pipe i the bottom

Storage is maintained for a minimum period of 48 hours while awaiting the results of the sterility tests after which all contaminated pulps are discarded This period has occasionally been extended to 10 days without deleterious effects on the virus content

Refrigeration

As dry ice (carbon dioxide snow) was not commercially available in Rio de Janeiro an industrial chilling machine was acquired from the *Deepfreeze International Corporation Chicago Ill* The model selected was the Cascade −120°F which is capable of maintaining a temperature of −85°C (−120°F) and at that temperature removing 1 000 BTU (British thermal units) per hour Any other make having the same characteristics will serve (Throughout the text this machine will be referred to as the deep freeze)

About 200 litres of alcohol as a convection fluid are kept cold in the chilling chamber of the deep freeze all the time The following adaptations to this deep-freeze are necessary

A ½ inch (13 mm) pipe is soldered to its bottom to allow for the drawing off of the cold alcohol

A small electric geared pump and a system of tubes are used to distribute the alcohol to the various apparatuses needing refrigeration The pump and tubes are covered by a thick layer of insulation The flow is governed by hand valves conveniently placed

Two circular openings are made through the lid of the cold chamber to permit the introduction of the condensers into the cold alcohol When not in use these openings are closed by insulated covers

The bulb of a recording thermometer is kept permanently in the alcohol

Thawing of pulps and centrifugation

The bottles containing sterile embryo pulps are immersed in water maintained at about 30°C until the contents have melted

In the dust proof room the bottles are opened and a pool is made by aspirating with a small vacuum pump all the pulps into a large filtering flask

From a side tubule at the bottom of the flask the pool is distributed into 250 ml centrifuge tubes These are then capped and spun for 1 hour at 2 000 revolutions per minute in a refrigerated centrifuge (2° 5°C)

The clear supernatant fluid (usually about half the volume of the pulp) is drawn off and constitutes the vaccine

Ampoules

Ampoules are made of Pyrex or similar glass and have a cylindrical body 15 mm in diameter and 45 mm long joined to a neck 8 mm in diameter The total length is 140 mm and the thickness of the glass is about 1 0 mm They are tested for leaks by subjecting them under water to an internal air pressure of 1 atmosphere Leaks are located by the escape of air bubbles and the defective ampoules are discarded After washing they are dry sterilized in special stands

The Standards recommend that a label containing certain information should be placed on each ampoule of vaccine This was not found practical because when ampoules are shipped in containers with ice these labels become damaged or come loose For this reason ampoules used in the Rio de Janeiro laboratory are stamped in the factory with the vaccine lot number in groups of 1 050 The marking is made permanent by the use of special ink and heating

Filling and plugging of ampoules

The flask containing the vaccine is connected to an automatic pipetting machine To the delivering end of this machine is attached a multiple jet device making possible the simultaneous filling of eight ampoules A hood is provided to protect this operation (fig 7 8 and 9)

B ewer utomatic p pett g mach e 120 or 40 (Balt mo B olog al Laboratory Inc Balt mo e Md USA)

FIG 7 FILLING AMPOULES — I

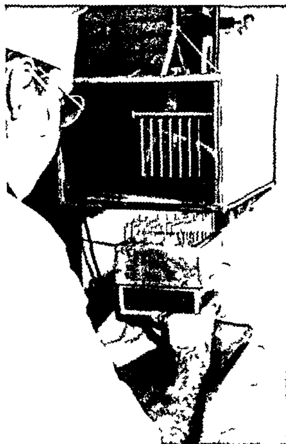


FIG 8 FILLING AMPOULES — II



Ampo les in the special sta ds a e filled from the m ltiple-jet device (see fig 9) co ected to the automatic pipetting machine

FIG 9 FILLING AMPOULES — III



The ope ato watches through a glass wi dow i the fro t of the hood The injecting device is made of stainless te l The openi gs at the e d of th tubes a e e y small re ult ng in a relatively h gh pr su e bei g c eated i side the instrume t at th moment of inject on and consequently in a distributi n of eq ual ol mes

The ampoules are arranged in specially made metal boxes or stands that hold them in upright position in eight rows of eight ampoules per row (fig 10) By pressing a foot switch a predetermined amount of vaccine is injected into the eight ampoules in one row When all 64 ampoules in a box are filled it is covered and moved on to the next operator

Another device has been developed to place sterile cotton plugs in the ampoules (fig 11) This is an apparatus designed to fit the top of the box containing the filled ampoules It has 64 upright metal tubes of a

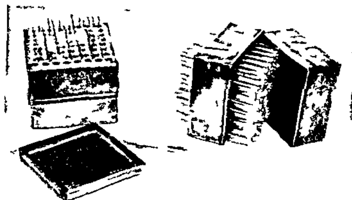
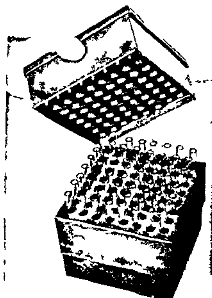


FIG 10 AMPOULES IN SPECIAL METAL STAND

The metal stand at the right has been opened to show the internal a angement

diameter slightly larger than the necks of the ampoules and so spaced as to fit these necks like sleeves as they stick out of their holding box The metal tubes contain other rimmed short glass tubes in which have been placed rather loose cotton plugs The glass tubes being shorter than the metal tubes allow for the tips of the ampoules to be introduced in the metal tubes and to come in contact end to end in perfect alignment with the glass tubes holding the plugs The whole is dry sterilized After this apparatus has been fitted to the box containing the ampoules the plugs in each row are pushed with an eight pointed comb-like instrument (fig 12) down into the necks of the ampoules to a depth of 1 cm from the tip (fig 13) This instrument is sterilized by occasional flaming

FIG 11 APPARATUS FOR PLUGGING AMPOULES



By the procedures just described which may be better understood by studying the illustrations two operators can aseptically fill and plug 1 000 ampoules in less than 15 minutes

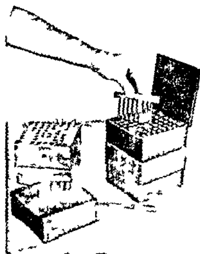
All these operations should take place in a dust proof room under the most strict aseptic con ditions

Shell free ing

The ampoules containing the vaccine are rotated in a specially designed machine which is kept partially submerged in cold alco hol pumped from the deep freeze (fig 14) The alcohol circulates continuously the correct level being maintained by an overflow pipe through which the excess is returned to the chilling chamber of the freezer

The ampoules rotate horizon tally and the frozen vaccine forms a thin walled hollow white cylin der occupying most of the body of the ampoule

FIG 12 PLUGGING AMPOULES — I



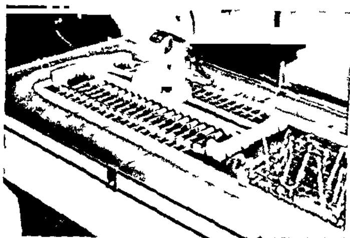
Cotton plugs being pu h d int the n ks of the ampo les by m a of a spec al n st ument

FIG 13 PLUGGING AMPOULES — II



Th cott n pl g a e hown pos tion in the ampoule

FIG 14 SHELL FREEZING OF VACCINE IN AMPOULES



Cold alcohol (–80 C) circulat ng from the deep freeze keeps the rotat ng machine partially subme ged

Desiccation

A two stage method of desiccation has been devised

First stage In the first stage the ampoules containing the shell frozen material are placed in a large metal desiccator A cold trap and a vacuum pump complete the system Vacuum is measured by a McLeod gauge (fig 15 16 and 17)

The desiccator is a hollow iron cylinder 18 inches (45 cm) in diameter 30 inches (75 cm) deep and $^{1}/_{8}$ inch (3 mm) in wall thickness A rim half an inch (12 mm) wide is welded to the upper edge and then machined flat A copper *tubing coil around the outside of the desiccating chamber* through which cold alcohol from the deep freeze may be made to cir culate provides the means for cooling it A layer of thermal insulation 3 inches (7 5 cm) thick covered by a thin metal sheet completes the wall of the cylinder Screws on the base provide a means for levelling

The lid of the d siccator is machined from a 1 inch thick (2 5 cm) Duralumin plate It has a circular groove fitted with a rubber ring to seal it against the rim of the desiccator Through an opening in the centre of the lid a metal tube 3 inches (7 5 cm) in diameter connects the desic cator to the cold trap Rubber washers smeared with a castor oil base lubricant keep the system vacuum tight The cold trap or condenser is essentially a hollow brass cylinder 8 inches (20 cm) in diameter and 28 inches (70 cm) in length The thickness of the wall is about $^{1}/_{16}$ inch

FIG 15 DESICCATING APPARATUS — I

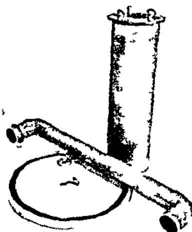


The des c to (ot n se) is show t the left The chilli g chamber of th deep f ez stands i th cent A syst m of m tal pip s w th ubber con e ions p o ides commu ic tion b twe n th old t p imme s d in th alcohol of the d ep-f eeze and th f e ma folds to d d with ampo les of acc ne in th s co d stage 1 desiccatio

(1 5 mm) The cylinder is closed at the bottom and has two openings in the top one for connexion with the desiccator the other opening is soldered to a brass pipe 1 inch (2 5 cm) in diameter extending almost to the bottom of the condenser and leading to the vacuum pump The vacuum pumps used are Hypervac No 25 manufactured by the Central Scientific Co of Chicago Ill Pumps of other makes having similar characteristics will serve

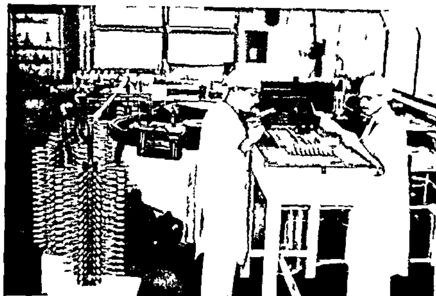
The temperature of the desiccator is first set to −10°C As soon as a reasonable vacuum is obtained there is no more risk of melting the vaccine and the temperature is allowed to rise slowly Desiccation proceeds overnight the vacuum pump being kept

FIG 16
DESICCATING APPARATUS — II



I r l wofld f desic ator (l ft) cond n e p c ld t ap u ed with de iccato (right) a d 3- ch (7 5 cm) m tal pip co n ct ng the two (c ntre)

FIG 17 GENERAL VIEW OF DESICCATING ROOM



One lot of vaccine is in the final stage of desiccation Another lot is being frozen in ampoules and being placed in the chilled desiccator (hidden by the deep freeze)

running continuously The cold trap is maintained at −80 C by immersion in the alcohol of the deep freeze The vacuum obtained is usually around 5 μ Hg

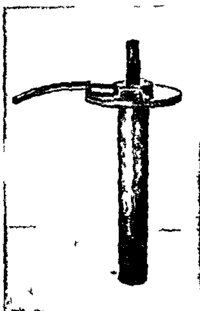
Second stage On the next day the desiccator is opened and the second stage of desiccation begins The vaccine as it comes out of the desiccator is already dry and the purpose of the second stage is to remove whatever moisture has been absorbed from the atmosphere when the desiccator was opened and to make possible the sealing of the ampoules without further exposure to atmospheric air In the second stage manifolds are used similar to those described by Bauer & Pickels but of larger capacity The cold trap is the same as that described by Bauer & Pickels [1]

The manifold also called tree is a copper tube 3 inches (7 5 cm) in diameter and 30 inches (75 cm) long mounted on a heavy metal base to give it stability when standing on end There are 204 outlets arranged radially around this vertical tube Each outlet is a piece of copper tubing $1^1/_4$ inch (32 mm) long with an outside diameter of $^5/_{16}$ inch (8 mm) hard soldered to an opening drilled in the main tube

The ampoules are attached to the outlets of the manifolds with short pieces of rubber tubing lubricated with castor oil Five manifolds will take care of one lot of vaccine of the usual size consisting of approximately 1 000 ampoules

Throu_h a system of metal tubes and rubber joints several manifolds can be connected to one cold trap (fig 18) A vacuum pump and a manometer of the McLeod type complete the system The pressure obtained varies between 1 μ Hg and 5 μ Hg The cotton plugs are not removed from the necks of the ampoules desiccation taking place through them

FIG 18 COLD TRAP OR CONDENSER FOR USE WITH THE MANIFOLDS IN THE SECOND STAGE OF DESICCATION



On the next day vacuum is broken by introducing dry nitrogen and the manifolds are clamped off and removed to a convenient place where the ampoules are sealed with a double flame gas oxygen torch (fig 19)

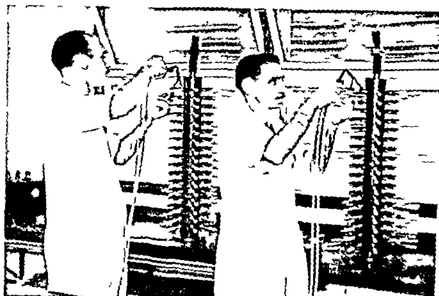
To avoid increase in pressure by the slight warming up of the nitrogen during the sealing operation an escape is provided through one of the outlets by attaching a flask containing a chemical desiccant The sealed ampoules are checked for defects and proper numbering counted and stored at −25 C

Sterility test

Fluid thioglycollate medium in dehydrated form is used for the sterility test Sixteen culture tubes are inoculated from the delivery tips of the automatic pipetting machine just before beginning distribution and 16 at the end Eight tubes from each group are incubated at 37°C and the other eight are left at room temperature The volume of vaccine inoculated into each culture tube is 0 5 ml Readings are made after one week and the test is considered satisfactory if no contamination appears If contamination appears in several tubes the lot is discarded If contamination appears in only one tube the test is repeated using the rehydrated contents of ampoules One tube of medium is used for each ampoule

The number of ampoules to be tested depends on the size of the lot and should be three for the first 100 ampoules and one for each additional

FIG 19 SEALING OFF AMPOULES FROM THE MANIFOLDS WITH CROSS FLAME BLOWTORCHES



50 ampoules If the original contamination shows up in any of the tubes the vaccine lot is discarded

If a different organism appears in only one of the tubes the test may be repeated

Guinea pig test

An extra check on the sterility is provided by the guinea pig test

Each of two guinea pigs is inoculated intraperitoneally with 4 ml of rehydrated vaccine undiluted The animals are observed for 15 days and their temperature is taken every day The lot is discarded if any significant reaction appears Short periods of fever are not uncommon and are not considered significant The test may be repeated if necessary

Virus concentration and expiration date

One mouse unit or LD_{50} is defined by the Standards as that quantity of yellow fever virus which when inoculated intracerebrally into each of an adequate number of yellow fever susceptible adult mice (16-20 g) will kill 50% of the animals due to a specific yellow fever virus encephalitis within 21 days The 50% end point is estimated by the method of Reed & Muench[8]

The concentration of virus in a suspension or titre may be expressed by a number corresponding to the dilution containing 1 LD_{50} in 0 03 ml An alternative way of expressing the titre is to state the number of LD_{50} in a specified volume

TABLE I TITRES OF 20 TYPICAL VACCINE LOTS PREPARED AT THE YELLOW FEVER LABORATORY INSTITUTO OSWALDO CRUZ RIO DE JANEIRO

Lot no	Before desiccation	After desiccation
171	1 183 000	1 102 000
172	—	1 91 000
173	1 157 000	1 67 000
174	—	1 95 000
175	1 377 000	1 90 000
176	1 70 000	1 55 000
177	1 183 000	1 53 000
178	1 372 000	1 68 000
179	1 205 000	1 80 000
180	1 191 000	1 70 000
181	—	1 205 000
182	—	1 188 000
183	1 242 000	1 195 000
184	1 219 000	1 111 000
185	1 202 000	1 84 000
186	1 191 000	1 45 000
187	1 294 000	1 203 000
188	1 282 000	1 262 000
189	—	1 205 000
190	1 311 000	1 250 000
	Mean 1 232 000	Mean 1 126 000

M [illegible] s calc lat d f om th ee 11 ti s on d f te ent mpo les

In titrating vaccines serial fourfold dilutions are prepared in 0 8/ NaCl solution containing 10/ non immune human or rhesus monkey serum Each dilution is inoculated into a group of 12 mice each animal receiving 0 03 ml intracerebrally The titre i e the dilution which kills *50/ of mice is thus determined*

The minimum amount of active virus for the satisfactory immunization of man was set by the Standards at 500 LD_{50}

In order to have a larger margin of safety when using vaccines in dilution of 1 100 1 000 LD_{50} was adopted in Brazil as the minimum

immunizing dose Consequently vaccines should have a titre of at least 1 6 000 at the time of administration which corresponds to approximately 200 000 LD_{50} per ml The virus concentrations obtained by the methods described are however much higher than the limits proposed as is shown in table I where the titres of twenty recent lots made in Rio de Janeiro are presented

Vaccines are titrated immediately after desiccation and at regular intervals thereafter At the time of shipping a vaccine to the field a few ampoules of the same lot are transferred from the cold storage (–25°C) to an ice box (+5°C) The conditions under which the vaccine is maintained in the field are thus approximately duplicated in the laboratory Monthly titrations are made from these ampoules By this procedure it is possible to follow the drop in virus concentration of all lots in the field and to advise the vaccination teams when the use of any lot should be interrupted

The methods of 17D vaccine preparation described above were developed having in mind the exigencies of mass vaccination of the rural populations of the South American continent and especially of Brazil Refrigeration is not available in many of the areas where demands for vaccination are most pressing

Difficulties in transport have been partially overcome by concentrating the vaccine so that one or two hundred persons can be immunized with the contents of a single ampoule

Desiccation for preservation of the virus is carried to a point where the residual moisture cannot be determined by the usual phosphorus pentoxide vacuum method As a matter of fact the dry vaccine gains weight when placed in a desiccator in the presence of pentoxide This extreme degree of desiccation justifies the use of the vaccine even after it has been kept without refrigeration for 15 days in places where no refrigeration is available Such conditions prevail in the Amazon region where several days travel by canoe are often necessary to reach the place of vaccination For this special type of work lots of vaccine having high virus titres are selected

Vaccine lots of high titre are also selected to be sent to foreign countries and an expiration date is set which corresponds to a period of six months from the date of issue provided the vaccine is kept at a temperature of 5°C or lower

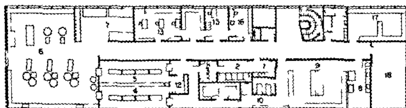
Laboratory

A laboratory for the production of yellow fever vaccine should be located in a building used exclusively for that purpose

A new yellow fever vaccine laboratory is now being built in the Oswaldo Cruz Institute which might serve as a model for other interested institutions

The layout of the upper floor where the vaccine is to be prepared is shown in fig 20

FIG 20
PLAN OF UPPER FLOOR OF RIO DE JANEIRO YELLOW FEVER LABORATORY





Eggs a e wa h d in o m 1 i cubated in 2 c ndi d 3 a d nocul t d om 4 5 (d t p oof oom) wh the emb yos a e also ha v sted and the v cin is d t ibut d in ampo les D icc t n t kes place i om 6 nd th finished v ccin is st ed in f ee e s in o m 7 Space is al o pro id d in oom 7 to packi g th ac e f shipm nt

Gl sswa e nd othe m s lla eous lab ato y mat al n ed ng st li at on a washed in oom 8 p epa d fo te ilizat o i 9 d t ill ed in to f es o o m i 10 The ste ili ed mat i l is the sto d in oom 11 p ndi g u a i oom 4 5

The ground floor is to be occupied by a mouse-colony room instrument repair shop storeroom and other accessory services

This new laboratory was planned for a monthly production of three million doses of vaccine about double of the present capacity of the Rio de Janeiro laboratory

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ADMINISTRATION OF 17D YELLOW FEVER VACCINE

With Special Reference to Brazil

H A PENNA M D

In cha ge of the Yellow Feve Labo oto y
In t tuto Oswaldo C u Rio de Jan o B a il

Vaccine is shipped in wide mouth vacuum bottles of one three or five gallons packed with cracked ice After every 48 hours the water should be drained off and the container repacked with ice

A letter accompanying each shipment of vaccine contains the necessary information such as conditions of storage volume of embryo fluid in the ampoules before desiccation and instructions for making the dilutions

The National Yellow Fever Service is in charge of the large scale admin istration of vaccine in Brazil

Vaccinating units usually composed of two trained vaccinators are sent out in accordance with an over all plan for a vaccinating campaign Transport usually Jeeps is provided by the Service Doctors are respon sible for supervising the activities of these units

Advance notices are posted advising the people to assemble at deter mined places on a specific date for vaccination

The technique of vaccine administration in the field somewhat modified from Smith Penna & Paoliello is as follows

Equipment for field unit

Except for the vacuum bottle in which the desiccated vaccine is packed in ice all the material required by a field vaccinating unit as listed below is packed in a simple specially designed packing case (fig 1 2 and 3)

2 white laboratory coats
4 linen towels
1 square piece of white oil cloth
1 Primus stove
1 alcohol lamp

Smith H H Pen a H A & Pa l llo A (1938) Am J t p M d 18 437

FIG 1 EQUIPMENT FOR FIELD VACCINATION PACKED IN SPECIAL CASE



On the left is the vacuum bottle in which the desiccated vaccine is packed in ice

FIG 2 TABLE ARRANGED FOR GROUP VACCINATION

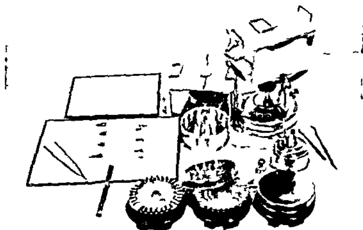
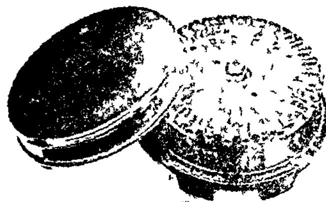


FIG 3 VIM FORSBECK NEEDLE RACK AND METAL BASE



2 metal sterilizers 16 16 10 cm (one for needle racks the oth r for syringes)
1 small flask (Erlenmeyer) 20 ml or 50 ml capacity with glass tub for covering
1 metal container for keeping vaccine flask iced
1 diamond pencil
15 metal tipped 10 ml syringes
4 needle racks and three bases for the Vim Forsbeck immunizing outfit [b]
93 needles 25 gauge ½ inch (0 50 mm × 13 mm) fitting in n edle rack
3 needles 19 gauge 2½ inches (1 10 mm 64 mm)
1 glass rod
12 glas tubes 4 cm in length
6 glass tubes 10 cm in length
Metal covers for needle racks
10-ml ampoules of steril saline (0 8 % NaCl)
1 litre 70 alcohol
Sterile absorbent cotton
24 rubber bands
1 forceps 12 cm

[b] Obtained from MacGregor Instrument Co Needham Ma s USA
Metric c nvers ons of needle spec ficat ons are not m thematically correct but correspond to actual d mens ons used

1 forceps 20 cm with ends bent for removing needle racks from sterilizer
1 white enamelled tray 32 × 25 × 4 cm
1 white enamelled tray 25 × 20 × 3 cm

Technique

On arrival at the building where a group of persons is to be vaccinated two tables should be arranged, one for the preparation of the vaccine and one for making the records

Where large groups are to be vaccinated printed slips of paper are distributed on which the desired information—name age sex—is entered for each person At the moment of vaccination each person presents his slip already filled out the slips are placed on a wire spike file in the order in which inoculations are made and the names may then be copied at leisure into the permanent record book in the same order

The materials are unpacked and arranged on the table selected for the preparation of the vaccine which is first covered with a white oil-cloth

One sterilizer containing the 10 ml syringes three long needles the short and long glass tubes and the vaccine flask is filled with water and boiled on the Primus stove When the water in the first sterilizer has boiled for five minutes the second sterilizer containing three needle racks with needles and covers is put on to boil In the meantime the other necessary items are laid out on the table Ampoules containing the desiccated vaccine are removed from the vacuum jar the number to be used depending on the number of people to be vaccinated the volume of vaccine in each ampoule and the dilution recommended

The three bases of the Vim Forsbeck outfit the fourth needle rack and ampoules of NaCl solution are placed on the table As soon as the materials in the second sterilizer have been boiled sufficiently two needle racks with needles are removed placed on their bases and covered The sterilizer is left on the stove so that the needle racks can be boiled as the needles are used To open the ampoules a scratch is made near the tip with the diamond pencil and the tip of the heated glass rod is applied With a sterile 10 ml syringe with a long needle the saline is added to each ampoule in such amount as to obtain a dilution of 1 10 If the ampoules contain the desiccate of 0 5 ml of vaccine the amount of saline to be added should be 5 ml The rehydration takes place almost immediately and with the same syringe and long needle the contents of all the ampoules are pooled and transferred to the small Erlenmeyer flask After being capped with the short glass tube the flask is kept chilled with ice in the metal dish designed for that purpose This first dilution should not be kept for more than 3 hours

When everything is in readiness to begin the inoculations the final dilution of the virus is made In order to make the 1 100 dilution of vaccine 9 ml of saline are drawn into the syringe Taking care to avoid loss of any saline through the needle exactly 1 ml of the 1 10 dilution is then sucked up into the saline The syringe is then rotated several times until the contents are thoroughly mixed This final 1 100 dilution should be used without delay The long needle is removed and a short one is taken from one of the needle racks for the first inoculation An area of the skin on the left arm of each person to be vaccinated is cleaned with cotton moistened with alcohol and 0 5 ml of the diluted 1 100 vaccine is injected subcutaneously near the insertion of the deltoid muscle After each inoculation the used needle is placed in the non sterile needle rack and a new one taken up from the sterile rack Thus the needle is changed for each person When the needles of the first rack have been used the second rack is brought into use The third needle rack which was boiling in the sterilizer is substituted for the non sterile rack on the third base Before placing the rack with the used needles back into the sterilizer it is *necessary to wash the needles one by one by forcing clean water through* them with a syringe

Each rack holds 31 needles When the first syringe which contains sufficient material for twenty persons is emptied a second one is filled in the same manner The recommendation for final dilution (usually 1 100) is the maximum permissible dilution but there is no contra indication to use of the vaccine in a lower dilution when small groups are to be vaccinated However concentrations greater than 1 10 should be avoided because of the possibility of allergic manifestations occurring in persons sensitized to chick protein

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cases of encephalitis have not been recorded in French territories after the use of a virus which is so highly neurotropic in laboratory animals Since it is very difficult to make an accurate follow up of vaccinated Africans [3] it is possible that in the French territories where neurotropic vaccine appeared harmless the methods of follow up employed were insufficient to bring to light the cases of encephalitis which actually occurred

Because of the hazards associated with the Dakar method Hahn [9] working in Nigeria and Dick [2] in Uganda independently investigated the use of 17D vaccine administered by scarification In the investigations reported by Dick 17D chick embryo vaccine prepared by the laboratories of the International Health Division of the Rockefeller Foundation New York was used Vaccinations were made at the site of the insertion of the deltoid after the area had been rubbed dry with cotton wool but without any other cleansing The skin was stretched tightly by grasping the arm firmly Two drops of the vaccine suspended in gum arabic or in saline were applied to the stretched area and two scarifications of about 10 mm in length were made through them There was no difference in the results whether gum arabic or saline was used to suspend the virus Gum arabic suspensions of the virus have however the advantage that there is less tendency for the vaccine to run down the arm than with water or saline suspensions The disadvantages of gum arabic suspensions are that they require constant mixing and if care is not taken to scarify immediately after applying the vaccine a film is formed which prevents the virus from making contact with the broken skin The scarifications were similar to those used in smallpox and of sufficient depth to cause a slight oozing The vaccinations were done in the shade and the vaccinated persons were kept in it for ten minutes after vaccination they were instructed not to wash the area for 24 hours In order to remind them not to do so a small loose dressing was placed over the area

Using this method Dick showed that of 91 adults with no demonstrable yellow fever antibody who were vaccinated by scarification through drops of vaccine containing approximately 8 000 intracerebral mouse LD_{50} of virus 85 (93 4%) developed sufficient antibodies in their sera to give positive results in neutralization tests made a month afterwards He further showed that 8 000 LD_{50} of virus was greatly in excess of the dose which would effectively immunize by scarification

The results of an experiment in which a comparison was made of the immunizing power of small doses of 17D vaccine administered by scarification or by inoculation are shown in the following tabulation in which the numerator is the number of persons with antibody 28 days after vaccination and the denominator is the number with negative prevaccination sera

Mouse LD50 placed on skin or inoculated subcutaneously	Results after scarification	Results after inoculation
3.2×10^3	3/3	[illegible]/2
3.2×10^2	[illegible]/2	3/3
3×10	2/[illegible]	1/[illegible]
3	1/3	1/3
0.3	0/2	0/2
0.03	0/3	0/3

Although the numbers are small it may be seen that with high dilutions there was one more positive in the scarification group than in the group inoculated subcutaneously with the same volume of vaccine as was placed on the arm In this connexion it is of interest to recall that Fox Kossobudzki & Fonseca da Cunha[8] showed that man was more susceptible to virus inoculated by the intradermal route than subcutaneously Although it would be valuable to have more figures on the susceptibility of human beings to small amounts of virus inoculated by scarification the data available show that under experimental conditions the virus is an efficient antigen even at high dilutions when administered by scarification

Hahn[9] carried out a large scale field trial of the scarification technique using 17D chick-embryo vaccine suspended in gum arabic solution This mixture was desiccated and could be satisfactorily rehydrated for dermal application He vaccinated 3 808 persons with his vaccine using a technique similar to that already described and showed that of 41 persons whose sera were negative 38 (92.7%) became positive as a result of vaccination and that of 68 samples taken at random from those who were vaccinated 65 (95.6%) were positive Even allowing for the natural immunity rate in the area where he was working the percentage of those who might be expected to have had negative prevaccination sera and of those who became positive after vaccination would be about the same

These findings of Hahn and of Dick have been confirmed by Cannon & Dewhurst[1] both with regard to the percentage of those with negative prevaccination sera whose sera became positive after vaccination and with regard to the observation that gum arabic is not an essential constituent of the vaccine From Cannon & Dewhurst s studies it would seem important that the skin at the site of the vaccination should not be cleansed with ether This procedure under certain conditions gave a lower percentage of positives Although ether inactivates yellow fever virus it is difficult to understand how this could have happened if the ether had been allowed to evaporate before applying the vaccine In any event there is no need to do more than rub the area dry with a cotton wool swab unless the vaccination site is grossly contaminated when cleansing with soap and water could be performed

In general the scarification technique with 17D virus has several advantages over subcutaneous inoculation of vaccine quite apart from the possibility that the virus is more antigenic when administered by scarification First in the preparation of vaccine for subcutaneous injection only the supernatant fluids of the infected embryo suspensions are used and about one third of the volume of the embryo suspensions is discarded This loss of potential vaccine virus would be avoided if the vaccine were given by scratch since the whole embryo extract could then be employed Secondly since the use of a sterile syringe for each person is usually impracticable in large scale vaccination doing without syringes altogether would prevent the transmission of disease by them A vaccine for scarification prepared by the method described by Hahn or by making a simple crude suspension of infected decapitated chick embryos would not require the strict bacterial sterility applicable to subcutaneous vaccine and would thus reduce costs in manufacture

The chief objections made to the scarification technique are (*a*) that one is less certain that virus will make contact with the cells than when virus is inoculated subcutaneously and (*b*) that the vaccine may be washed off by uncooperative recipients There appears to be no reason why multiple scarifications should not be made over each drop of vaccine such a procedure would render more susceptible cells available for infection and the multiple puncture method as applied to vaccination with vaccinia virus might ensure with even greater reliability infection with the virus

Vaccination by scarification with crude whole chick embryo suspensions would seem to be administratively and financially practicable on a mass scale in endemic zones Since it is possible that smaller amounts of virus are antigenic when administered by scarification than by subcutaneous inoculation of the same volume of vaccine it would seem important that a large scale study of the immunity produced by scarification using about 100 mouse intracerebral LD_{50} of virus should be investigated If this dose were found to be adequate then it would be possible to use a 5 ml ampoule of *intracerebral titre as low as* 10^{3} *per* 0 03 *ml* to vaccinate more than 1 000 people with 100 LD_{50} for each scarification This would produce a further tenfold reduction in the cost of mass vaccination

If it is found that the production of crude chick embryo vaccine is more expensive than mouse brain vaccine attempts should be made to investigate the antigenicity of 17D mouse brain virus as a scarification vaccine This might be expected to be much less dangerous than French neurotropic *mouse brain vaccine* but experiments are required to establish this in man Two objections to the use of mouse brain vaccines are generally raised

Since the preparation of this *manuscript* D A Cannon and F Dewhurst (*Ann trop Med Parasit* 1955 49 174) *have described a method of preparing* 17D virus yellow fever vaccine in mouse brain and the results of a human trial with their vaccine

First the vaccine may be contaminated with a latent mouse virus which is pathogenic for man The only virus of this group which requires consideration is lymphocytic choriomeningitis (LCM) virus There would seem to be no difficulty in keeping a stock of mice which are to be used for vaccine production free from LCM virus Secondly Smithburn [16] has suggested that there is a potential hazard of allergic demyelinating encephalomyelitis when mammalian tissue is used for vaccine It is very doubtful whether this could ever occur with the amount of nervous tissue which would be placed on the skin surface for vaccination by scarification These objections to the use of 17D mouse brain vaccine are more academic than real If 17D mouse brain vaccine is antigenic when administered by scarification—which is likely—and if it does not produce encephalitis in man then mouse brain infected with 17D virus might be used with advantage for vaccination by scarification

The use of a combined 17D yellow fever and vaccinia vaccine was investigated by Hahn [9] and by Dick & Horgan [4] Hahn found that 12 out of 12 persons vaccinated with a yellow fever and vaccinia vaccine had developed yellow fever antibody by the sixth week after vaccination Dick & Horgan however in a small completely controlled experiment found that only 14 out of 21 of those with negative prevaccination sera who were scarified with a mixed vaccine had developed antibody by that time It is possible that the differences in the results of Hahn and of Dick & Horgan using a combined vaccine were due to the relative titre of yellow fever and vaccinia viruses in their preparations While there is no evidence that vaccinia interferes with 17D yellow fever virus when the vaccines are given separately the results of Dick & Horgan suggest that there is a local inhibition whereby the presence of vaccinia prevents invasion by 17D virus Quite apart from this finding it is doubtful whether it is advisable to administer the two vaccines at the same time in view of the danger of severe reactions Peltier [14, 15] states that when French neurotropic yellow fever and vaccinia vaccines are administered by scarification the reactions are of the same magnitude as those observed after the use of French neurotropic vaccine by itself but that reactions are observed less frequently in the coloured than in the white races While Hahn and Dick & Horgan observed no reactions after the use of combined 17D yellow fever and vaccinia vaccines it should be noted that their studies were made in Africans and it may be that in other races the reaction to a combined or double vaccination might be severe Thus two mishaps have been recorded in Great Britain in association with the use of a combined 17D yellow fever and vaccinia vaccine and it would appear wise to follow the recommendations of the Ministry of Health London with regard to the period which should elapse between smallpox and yellow fever vaccination to ensure the maximum immunization and freedom from sequelae These are given in Annex 1

Annex 1*

INOCULATION AND VACCINATION OF TRAVELLERS

Many countries insist that travellers before entering their territories should produce satisfactory evidence of having been recently immunized against certain diseases particularly smallpox and yellow fever Inoculation against yellow fever is required of travellers who have come from or passed through an endemic yellow fever area when they arrive by air in a country where although the disease does not exist there may be conditions which permit of its development

In order to avoid detention or delays at the frontiers of countries requiring these measures travellers must provide themselves with certificates of vaccination or inoculation on the appropriate prescribed international forms

The Ministry of Health is often asked for advice as to the period which should elapse between smallpox vaccination and yellow fever inoculation to ensure maximum immunization and freedom from sequelae A meeting of experts [**] was therefore recently convened to review this subject and the following is a brief summary of their conclusions which should be considered as the official advice to those concerned viz

(1) That whenever possible yellow fever inoculation should precede primary vaccination against smallpox

(2) That there should be an interval of at least four days between yellow fever inoculation (when given first) and primary vaccination against smallpox (when given subsequently)

(3) That if primary vaccination against smallpox is done first there should be an interval of 21 days from the date of the vaccination before the yellow fever inoculation is given [When infants under the age of 9 months are to be vaccinated against both yellow fever and smallpox there should be an interval of 21 days between the two vaccinations no matter which is performed first]†

(4) That where there is evidence of previous successful vaccination against smallpox yellow fever immunization and re vaccination against smallpox may be carried out at the same session but if time permits yellow fever immunization should always precede re vaccination by at least four days

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* Reproduced from the *Monthl Bullet n of the M nistry of Health and the Publ c H alth Laboratory Se vice* 1949 8 36-37 by kind permission of the editors

[**] The composit on of the panel of experts was W H Bradley D M M R C P (Chairman) Ministry of Health J C Broom O B E M D *Wellcome Research Institution* E T Conybeare M D F R C P Ministry of Health Brig H T Findlay M B Director of Pathology War Office Air Cmdre J Kilpatrick O B E M B Director of Hygiene Air Ministry F O MacCallum M D Virus Reference Laboratory M R C D McClean M B Lister Institute W D Maycock M B E M D *Blood Transfusion* Service Ministry of Health Surg Cmdr E J Mo kler M B *Medical Department Admiralty* *L H Murray O B E M D* Ministry of Health H J Parish M D F R C P Wellcome Research Laboratories Brig A E Richmond O B E M R C S K H S Director of Hygiene War Office P G Stock C B C M G C B E F R C P Medical Adviser on International Quarantine Ministry of Health and G S Wilson M D F R C P Director Publ c Health Laboratory Service M R C

† Th s amendment to the official advice to travellers will shortly be introduced in the *Notice to Travell rs* issued by the Min stry of Health *London* and the Department of Health for Scotland Ed nburgh

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TIME OF APPEARANCE AND DURATION OF IMMUNITY CONFERRED BY 17D VACCINE

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Time of Appearance of Immunity

In 1931 Berry & Kitchen described their observations on seven patients who had accidentally contracted yellow fever in the laboratory and all of whom recovered from mild or severe infection By intracerebral inoculation of mice they were able to demonstrate virus in the blood during the first four days of illness and even on the fifth day (107 hours after onset) During the first four days 24 mice were inoculated with the patients blood and 12 mice on the fifth day and the mortality rate among the mice fell from 100% with first day blood to 0 with fifth day blood In the second part of their study these authors investigated the appearance and production of antibody in their patients serum Previous research by Hudson [11] had shown that the serum of patients protected monkeys against a test dose of virus from the fifth day of infection on With the mouse as test animal and using the test of Sawyer & Lloyd [13] Berry & Kitchen demonstrated virus neutralizing antibody in the serum of their patients from the fourth day of infection The proportion of mice protected rose from 0 on the third day to 100% on the fifth It was thus shown that yellow fever virus and the corresponding antibody could be present simultaneously in the blood on the fourth and fifth day This co existence of virus and antibody had already been observed with vaccine virus Smith [16] using a special technique had shown that antibody could be present in rabbit serum as early as the second day and virus as late as the eighth day Berry & Kitchen used a technique similar to Smith s to demonstrate this co existence in yellow fever The difficulties met by Smithburn Haddow & Lumsden [19] who were unable to infect one monkey from another naturally infected monkey but who did succeed in isolating virus in mice were no doubt due to the co-existence of virus and antibody in the monkey s blood

Theiler & Smith [21] in 1937 studied the appearance of antibody in eight rhesus monkeys Two monkeys were given subcutaneous inoculations

with infective mouse brains prepared from mice inoculated with the 216th subculture of 17D virus (73 000 000 mouse minimum lethal doses (MLD)) the six other monkeys received decimal dilutions of the supernatant fluid of the 217th subculture corresponding to 13 000 0 13 MLD From the results of these experiments it appeared that even minimal quantities of virus could produce infection and consequently the production of antibody and immunity Intracerebral inoculation of a test dose of French neurotropic virus showed that effective immunity was built up in the rhesus monkey as early as five days after inoculation (one monkey inoculated five days after the first injection had a febrile reaction but survived) The four monkeys which were given test inoculations 7 14 days after the first inoculation survived and their blood contained no virus during the six days following the test inoculation

Before using the rhesus tested virus for human vaccination the respons was tested of four immune persons to subcutan ous inoculation of virus derived from the 227th subculture on chick-embryo tissue One of the tested persons had had natural infection and the others had previously been vaccinated the first had an antibody titre of 1 32 before inoculation while the antibodies had almost disappeared from the sera of the three others All four showed marked serological response after inoculation Six non immune persons were later inoculated with a 50 000-3 000 000 mouse MLD of 17D vaccine After one week three sera showed no anti body but two weeks after vaccination demonstrable antibody was present in all six sera The antibody titre was generally very low

In a study published in 1938 Smith Penna & Paoliello [15] described the early use of yellow fever vaccination in Brazil Human vaccination with 17D vaccine was first undertaken on groups of volunteers in February 1937 Virus was most commonly found in the blood on the fifth sixth and seventh days after vaccination During the first 28 days after vac cination protective antibody was d monstrated by the mous protection test in all vaccinated persons None of the persons tested was immune 7 days after vaccination and 9 persons out of 23 showed no demonstrable antibody after 14 days The antibody titre was generally highest between the 21st and 28th day but in certain persons it continued to increase until the 70th day The serological response varied from one person to another and the titres were generally low compared with those in vaccinated rhesus monkeys or in persons recovering from naturally acquired yellow fever

In June 1937 after more than 100 persons had been vaccinated in the laboratory vaccination was undertaken in the coffee plantations of the Municipio of Varginha (State of Minas Gerais) For the first vaccinations only persons over 12 years of age were inoculated but the age limit was later lowered to 8 and then to 2 years Antibody appeared in the blood between the 7th and 21st days The percentage of immune persons after

vaccination was 97 (622 positive results out of 641 sera examined) As a result of vaccination undertaken later on an even larger scale in rural areas that number rose to 95/

In a study published in 1943 on the use of various strains of 17D virus as vaccine Fox & Penna[10] examined the correlation between the dose of virus inoculated the presence of virus in blood and the immunological response in monkeys subcutaneously inoculated With the highest doses there was virtually no circulating virus while antibody was demonstrable by the 7th day With lower doses virus did not appear in the blood until the 6th or 7th day and antibody was only demonstrable after the 11th day With the smaller inoculum the antibody level was higher than with the larger Although this relation between the dose of virus inoculated and the level of antibody obtained was not confirmed by a second series of tests with a different virus strain the results do indicate that there is a direct relationship between a virtual absence of virus in the circulation and a poor immune response These experiments also showed that intra cerebral inoculation of the monkey produces more circulating virus and a higher antibody level than subcutaneous inoculation

Carrying out mass vaccination in Brazil on some 500 000 persons between September 1937 and July 1938 Soper & Smith[20] observed that the vaccination became effective after one week

Smithburn & Mahaffy[19] systematically reviewed these experiments with a view to determining the time of development of immunity in vaccinated monkeys and published their results in 1945 They carried out over 16 000 tests using the Sawyer & Lloyd technique but found that that tech nique was not so suitable to the conditions prevailing in Uganda as to those in Brazil They obtained too great a proportion of inconclusive results with sera from zones where yellow fever occurred and with sera from vaccinated population groups Smithburn[17] devised a more sen sitive method which made use of 1/ mouse virus and which did not require more than 0 5 ml of serum and could be used either in adult mice previ ously prepared by an injection of starch solution or in normal 14 day old mice This technique which was better adapted to conditions in Uganda is as specific as the standard technique

It was using this test that Smithburn & Mahaffy conducted their study on the time of development of antibody after vaccination Twelve rhesus monkeys were given the standard dose of vaccine for man 0 5 ml of 1 10 dilution of rehydrated virus Protective antibody was demonstrable *in the* serum six to seven days *after vaccination It is interesting to note* that one monkey which received a test inoculation of pantrophic virus five days after vaccination survived A resistance had thus been acquired before circulating antibody could be demonstrated serologically It is

possible that other protective factors take effect before antibody becomes demonstrable

The same method was used for the study of development of antibody in man after vaccination Ten African volunteers were vaccinated with 10 000 mouse MLD and were then bled 7 10 14 and 21 days after vaccination One person showed antibody on the 7th day nine on the 10th day and all the sera were protective by the 14th day

Thus *man seems unable to form antibodies so early as the rhesus but* by the 10th day his serum has a very high degree of protectivity Judging from the studies carried out on monkeys it seems probable that a protective mechanism begins to operate in man by the 8th or 9th day

Duration of Immunity

With the introduction of various methods of vaccination the question arose of the duration of the immunity induced Smith Penna & Paoliello [35] in 1938 demonstrated the efficacy of 17D vaccine one year after inoculation and noted a fall in antibody level during that time and a considerable variation between individuals

Several years later in 1945 Smithburn & Mahaffy [39] in post vaccination studies in a coastal region of Kenya and in Bwamba County in western Uganda showed that the period of effectiveness might be as high as three years In the first mentioned zone 355 000 persons were vaccinated between April 1941 and May 1942 while in the second some 145 000 persons were vaccinated between May and August 1941 Serological examinations carried out two or three years after vaccination on representative groups of the vaccinated population showed that at least 90% were still immune The incidence of immunity had not declined during the third year The percentage of children immunized was generally as high as that of adults and the antibody level was maintained equally well in both Detailed results showed that sometimes adults and sometimes children showed the higher percentage of immunity Thus in the Bubandi area of Bwamba County 86 7% of vaccinated children were immune after three years and 93 3% of adults while in the Hakitengya area the figures were 100% and 94 3% respectively

On the basis of large scale studies Fox & Cabral concluded in 1943 that children could not be so easily immunized as adults and that they gave a lower antibody level which fell more rapidly In the course of their studies on post vaccination immunity with different strains of living 17D virus these authors examined 926 persons composed of several groups vaccinated during a period of four years Sera were collected at

different intervals after vaccination and were examined by the mouse protection test The results obtained showed that there were marked differences in the duration of immunity which were due not to variations in the antigenicity of different virus strains but to the age composition of the vaccinated groups Thus only 2% (4 sera) of the sera collected four years after vaccination in the groups composed mainly of adults showed any sign of protection On the other hand in sera collected three years after vaccination in groups composed chiefly of young persons approximately 10% (36 sera) showed no protective power The detailed analysis of results by age group showed with one exception a clear relationship between age and the immune level both one month after vaccination and at later intervals In their first conclusions the authors considered that age was an important factor in determining both the degree of serological response and the duration of immunity in vaccinated persons Although the examination of negative sera with a more sensitive technique showed detectable antibody in 61 8% of 139 sera tested it seems that in certain persons immunity could disappear altogether The work of these authors showed that a virus of known antigenicity conferred an immunity which was satisfactory from the group standpoint for four years and probably for longer since the antibody level decreases very slowly

In earlier studies Sawyer[1] had noted that vaccinated persons showed their highest antibody level during the six weeks following vaccination and that that level decreased gradually following a curve which tended to become horizontal approximately four years after vaccination The level of immunity acquired by vaccination was however lower than that conferred by natural contraction of the disease

Bugher & Gast-Galvis[3] showed in 1944 that the vaccination could continue effective for four years that the percentage of positive results was as high in children as in adults and that the immunity acquired lasted as long in children as in adults Anderson & Gast Galvis[1] in 1947 considered that the percentage of persons with positive results was the same five years after vaccination as after a few months whatever their age The percentage positive however increased with age from 91% at 6-9 years of age to 96% in persons over 30 years

In 1943 Fox Kossobudzki & Fonseca da Cunha[9] calculated the efficacy of vaccination on groups of fifteen children 5 20 years old Six mice were used for each serum tested or 90 mice for 15 tests in each group The numbers of surviving mice in each group of ninety were compared and a percentage for each year of age was thus calculated Comparison of the percentages obtained in this manner for the different groups led to the conclusion that the immune response increased with age up to 14 years However this method of collection based as it is on relatively small differences is not convincing Using more sensitive tests the same authors[8]

revised their conclusions several years later and considered the age differences not to be statistically significant. They found that with the more sensitive test only 4 of the 46 sera considered negative at the first examination in fact contained no antibody.

Dick & Smithburn,[6] in 1949, did not confirm the results of Fox & Cabral according to which the immune response to vaccination and the duration of immunity were lesser in children than in adults. The results of their own experiments were rather closer to those of Anderson & Gast Galvis. In their opinion, no distinction should be made between children and adults in the regulations regarding yellow fever vaccination. They also showed that a high percentage of vaccinated persons retained humoral immunity more than six years after vaccination, and they suggested that the validity of vaccination certificates should be extended from four to six years after vaccination with a vaccine of recognized potency.

In 1952 Dick & Gee[5] published the results of a post-vaccination survey carried out in the Toro district of Uganda. Three of the places chosen for this study lay within the area in which systematic vaccination had previously been carried out and the fourth lay in a zone where no vaccination had been done. In the first three places 77.2% of the persons tested were immune after nine years and over, while 17.5% were immune in the unvaccinated zone. Their results indicated that the period of validity of vaccination could be extended to nine years for persons over seven years of age at the time of vaccination.

Courtois[4] *in 1954 published the results of a post-vaccination study of* the duration of immunity conferred by vaccination of African patients from Ango (Uele, Belgian Congo) and neighbouring villages. In July 1953 sample sera were collected from 79 men whom administrative documents showed to have been vaccinated in 1941. The protection test was carried out by the Smithburn technique[17] using mice 5-6 weeks old prepared with an intracerebral injection of starch solution given almost immediately before the intraperitoneal inoculation. These mice were given 0.6 ml of a mixture of 1.5 ml of a 1/ suspension of virulent mouse brain and 3 ml of the serum under test. The following results were obtained:

Sera tested	*Positive*			*Doubtful*	*Negative*
	6/6	5/6	5/5	4/6	1/6
79	69	3	4	1	2 *

The denominator indicates the number of mice inoculated, and the numerator the number of mice living 4 days after inoculation and surviving until the 14th day.
* Of which one had been diluted by half.

Immunity was thus maintained in 76 of 79 patients tested, or 96.2%, for nearly 12 years. This finding confirms those of Dick & Gee who had proposed that the duration of the validity of the vaccination certificate

should be extended to nine years It should be noted that at the First Inter Am rican Congress of Public Health held in Havana Cuba in 1952 de Souza Manso [14] stated without comment that among the sera of 117 vac cinated persons tested 93/ remained positive 12 years later

As Mahaffey stated at the African Seminar on Yellow Fever h ld in Kampala Uganda in 1953 It is perhaps not too much to hope that it will eventually be found that the immunity following vaccination is life long

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MASS VACCINATION



MASS YELLOW FEVER VACCINATION IN FRENCH AFRICA SOUTH OF THE SAHARA

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Historical Background

The first experiments in large scale vaccination against yellow fever in French Africa south of the Sahara date back to 1934 when Laigret [3] came to the West African territories to try out an immunization process which he had just developed at the Institut Pasteur at Tunis

This method involved the use of the French strain of yellow fever virus modified by adaptation to the white mouse Three different batches of virulent brains after exposure to laboratory temperature for four days two days and one day respectively were ground up with sodium phosphate and then dried Vaccination involved the subcutaneous injection of each of the three batches at intervals of three weeks During 1934 and 1935 5 699 persons in French West Africa were given the three successive inoculations

This immunization process requiring the bringing together of the subjects to be vaccinated on three different occasions was difficult to apply in large communities Nicolle & Laigret [4] therefore simplified the method in 1935 using a single injection of virus exposed for 24 hours to a temperature of 20 C and then coated with a layer of egg yolk and dried This new method was adopted at the beginning of 1936 by the Institut Pasteur at Dakar which up to the end of 1939 prepared and supplied sufficient vaccine to immunize about 15 000 persons

In 1939 Peltier et al [5] trying to find a simple and practical method of inoculation observed that when a drop of virulent mouse brain suspension was deposited on scarifications made in the skin of fresh *Macacus rhesus* monkeys the yellow fever virus multiplied in the blood of the animals and that immunity to yellow fever took the same time to develop as in the case of subcutaneous injection By using the same method to carry out inoculations with a mixture of mouse adapted yellow fever virus and

smallpox vaccine these workers also showed that it was possible to vaccinate simultaneously against yellow fever and smallpox

The first trials on human beings were carried out shortly afterwards on a few volunteers and confirmed the results obtained with monkeys Subsequently after successfully applying this m thod to several hundred individuals from readily supervised communities (soldiers and school children) Peltier et al [8] in agreement with the authorities applied this mixed vaccination technique on 100 000 Africans in Senegal

These op rations were carried out in 1939 in three successive periods lasting 36 days in all during the months of May June and July the hottest months of the year In each locality visited vaccination sessions took place as described below

Three folding tables were set up in the open air in the shade of a tree or of a shelter of millet stalks rapidly erected by the villagers or where they existed in premises better protected against the rays of the sun such as schools dispensaries or markets The session began with the collection of blood samples for prevaccination tests from the schoolchildren a list of whose names had been drawn up in advance The inoculations were then carried out

At one of the tables one operator was engaged solely in the preparation of the vaccine suspensions while the teams responsible for the actual vaccination were seated at the other two tables One hundred doses each of yellow fever and smallpox vaccine were ground up and mixed while dry and then made into a suspension with gum arabic solution The suspension thus obtained was poured into a watch glass and handed to the vaccinators (three per table) who were provided with several thousand vaccinostyles The time necessary to prepare two suspensions of 100 doses each was about the same as that required for each team to vaccinate 100 persons Thus the vaccinators were kept constantly supplied with vaccine and no interruptions were necessary In this way it was possible to carry out some 5 000 vaccinations per day at the rate of 800 per hour

Blood samples for testing the effectiveness of the yellow fever vaccination were collect d one to two months later Using the lists of names most of the subjects who had supplied the first samples before vaccination could easily be found again Of a total of 1 387 schoolchildren whose serum was shown to give no protection before inoculation 1 336 (96 %) later had a positive sero protection test

The possibility of the mass application of the yellow fever vaccination method suggested by the Institut Pasteur at Dakar having thus been demonstrated the Governor General of French West Africa laid down that this method should be systematically employed in active endemic foci and in densely populated areas from the b ginning of 1940 Detailed

instructions on the use of the vaccine the method of inoculation and the precautions to be observed to ensure the success of the operations were sent to all medical posts

The following year a departmental order made yellow fever vaccination by scarification compulsory for the whole civil and military population of French West Africa Since then mobile health service teams have applied this technique over an ever increasing area and despite material difficulties encountered during a period deeply disturbed by international events more than 15 000 000 inoculations had been carried out by the end of 1945 in the various territories of French West Africa

After the end of hostilities the progress made in mass vaccination against yellow fever was made known to the international health organizations meeting in New York The Quarantine Commission of the United Nations Relief and Rehabilitation Administration (UNRRA) then decided to have experiments carried out to assess the value of the Dakar vaccine and laid down the manner in which this study was to be conducted

The experiment took place in 1945 at Frejus in the department of Var France on 600 European soldiers divided into three groups of 200 each as follows

(1) Group A inoculation by scarification with Dakar yellow fever vaccine only

(2) Group B inoculation by scarification with Dakar yellow fever vaccine combined with smallpox vaccine

(3) Group C inoculation by subcutaneous injection of 17D vaccine

Blood samples collected a month later from the subjects inoculated were examined by three different laboratories (Dakar Montana and Rio de Janeiro) The results of the sero protection tests showed that immunity against yellow fever had been acquired in 98.9% of cases in group A 97.9% in group B and 64.2% in group C [1]

In view of these results the UNRRA Standing Technical Committee on Health decided to recognize the validity of the yellow fever vaccine of the Institut Pasteur Dakar

Yellow fever vaccination by scarification whether simple or mixed has steadily increased in French Africa south of the Sahara since then The health services of French Equatorial Africa and of Cameroon which adopted the Dakar method in 1944 and 1945 respectively have since then carried on uninterrupted systematic vaccination campaigns against yellow fever By 31 December 1953 the total number of inoculations performed throughout the territories of French Africa south of the Sahara exceeded 56 000 000 or more than double the population which may be assessed at approximately 25 500 000 Thus each inhabitant is assumed to have

been vaccinated twice Table I shows the annual distribution of these inoculations

TABLE I YELLOW FEVER OR COMBINED YELLOW FEVER AND SMALLPOX VACCINATIONS CARRIED OUT IN FRENCH AFRICA 1939-53

Year	French West Africa	Togoland	Cameroon	French Equatorial Africa	Total
1939	101 633	—	—	—	101 633
1940	357 581	15 051	—	—	372 63?
1941	1 498 689	45 600	—	—	1 544 689
1942	2 779 984	133 130	—	—	2 913 114
1943	2 858 312	100 037	—	—	2 958 349
1944	3 475 609	[illegible] 43	—	185 224	3 872 0[illegible]6
1945	750 824	234 999	360 823	90 228	2 436 872
1946	2 4[illegible]6 814	189 906	323 703	143 605	3 084 032
1947	7[illegible]0 505	338 932	649 661	885 438	4 644 536
1948	3 470 930	533 387	433 494	7[illegible]9 511	5 217 317
1949	4 131 943	236 710	118 996	794 588	5,282 237
1950	3 578 033	289 877	119 840	1 017 643	5 005,393
1951	3 861 802	397 193	879 726	880 757	6 019 478
195[illegible]	5 0[illegible]0 607	286,228	241 464	737 481	6 285 780
1953	4 071,539	224 090	322 406	720 844	5 338 878
Total	43 154 804	3 236 778	3 450 117	6,235 317	56 077 016
Population	17,300 000	1 000 000	3 000 000	4,200 000	25 500 000

Vaccination Results in French West Africa and Togoland

In order to determine the influence of mass vaccination on the prevalence of yellow fever in French West Africa a comparative list of the cases notified throughout the African continent during the last 20 years has been drawn up This list is reproduced in table II which shows the annual number of yellow fever cases recorded in the French West African territories and in the remainder of the continent Figures showing the increase in the number of yellow fever vaccinations since 1939 when they were started are also shown

An appreciable decrease in the prevalence of the disease in French West Africa after 1943 is at once apparent despite the fact that at that time mass vaccination had not yet covered half the population But it must be borne in mind that it was applied in the first place to the inhabitants of areas suspected of harbouring the yellow fever virus in order to prevent the spread of the virus to neighbouring regions

The majority of yellow fever cases recorded since 1944 have occurred in small isolated bush-communities such as plantations wood-cutters camps and railway construction camps their appearance has in no way coincided with the introduction of the virus by man but is certainly directly

TABLE II YELLOW FEVER VACCINATIONS AND CASES OF YELLOW FEVER IN AFRICA 1934-53

Year	Number of yellow fever vaccinations by scarification in French West Africa and Togoland	Cases of yellow fever notified		
		French West Africa and Togoland	Other African territories	Total for Africa
1934		23	41	64
1935		12	16	28
1936		24	19	43
1937		48	122	170
1938		27	49	76
1939	101 633	15	43	58
1940	372 632	4	4	8
1941	2 018 954	17	19	36
1942	4 932 068	10	6	16
1943	7 890 417	12	20	32
1944	11 577 269	2	11	13
1945	14 563 092	1	17	18
1946	17 179 812	1	51	52
1947	20 289 249	3	1	4
1948	24,293 762	2	4	6
1949	28 662 214	0	37	37
1950	32 530 124	0	17	17
1951	36 789 119	2	39	41
1952	42 095 954	1	53	54
1953	46 391 582	2	28	30

Cases occurring during the 1940 epidemic of yellow fever in the Sudan are not included

related to the presence of reservoirs of sylvatic virus in the immediate neighbourhood

Yellow fever cases have been classified in table III according to their variety urban or rural It will be seen that there was first a progressive decrease in the number of urban yellow fever cases and that no cases have been recorded in the last six years It may thus be assumed that the first result of mass vaccination combined with *Aedes aegypti* control has been the disappearance of this variety of yellow fever

In order to show up still more clearly the difference between the position of yellow fever in French West Africa and Togoland and that in the other African territories the data given in Table II are shown in condensed form in table IV The 20-year interval between 1934 and 1953 has been divided into four equal periods for each of which the prevalence of yellow fever in French West Africa and Togoland has been compared with the prevalence over the whole continent

TABLE III CASES OF URBAN AND RURAL YELLOW FEVER IN FRENCH WEST AFRICA AND TOGOLAND 1934-53

Year	Ca es of yellow fever		
	urban	rural	total
1934	20	3	23
1935	6	6	12
1936	15	9	24
1937	37	11	38
1938	21	6	7
1939	11	4	15
1940	1	3	4
1941	9	8	17
1942	2	8	10
1943	2	10	12
1944	1	1	2
1945	0	1	1
1946	0	1	1
1947	3	0	3
1948	0	2	2
1949	0	0	0
1950	0	0	0
1951	0		2
1952	0	1	1
1953	0	2	2

It will be seen that the an nual average of yellow fever cases in Africa fell during the second and third periods and subsequently increased appre ciably whereas in French West Africa the average has steadily decreased falling finally to one case only during the last five year period Furthermore the percentage of cases in French West Africa and Togoland among the total number of ca ses throughout Africa which was 35 1% before mass vaccin ation has subs qu ntly fallen to 2 7%

By 1951 there had been twice as many inoculations against yellow fever carried out in French West Africa and Togoland as there were inhabi

TABLE IV COMPARATIVE PREVALENCE OF YELLOW FEVER IN ALL AFRICA AND IN FRENCH WEST AFRICA AND TOGOLAND 1934-53

Five yea period	All Africa		French West Africa and Togoland		French West Africa and Togoland as p rcentage of all Afr ca
	numbe of cases	annual a erage	number of cases	annual average	
1934-38	381	76.2	134	26 8	35 1
1939-43	150	30 0	58	11 6	38 6
1944-48	93	18 6	9	1 8	9 6
1949-53	179	35 8	5	1 0	2 7

Cases occur ng during the 1940 epidemic of yellow fever in the S dan are not included

tants Thus in principle every inhabitant had been vaccinated twice How ever the five cases of yellow fever observed since then have proved that some persons escaped systematic vaccination despite the efforts of the mobile teams for whom the absence of precise census figures makes it very difficult to gather together all the inhabitants of villages scattered through

the African bush Since 1950 mass yellow fever vaccination in French West Africa has been *organized in such a way* as to cover a quarter *of the* population of each of the territories every year with the aim of maintain ing the l vel of protection already acquired and of safeguarding babies born in the intervening period It is hoped in this manner to reduce the number of defaulters to a minimum

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MASS VACCINATION AGAINST YELLOW FEVER IN BRAZIL, 1937 54

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After the discovery of the jungle form of yellow fever in Brazil in 1932 it became clear that this disease could not be fought simply by destroying the vector mosquitos or other carriers of the virus

Even before that date—in 1931—attempts had been made using an effective vaccine [2, 3] *to immunize all laboratory staff exposed to the risk* of contracting yellow fever as a result of handling highly infective material

We shall not go into the details of the various types of virus vaccine and their methods of use but shall restrict ourselves to a few remarks on the use of 17D virus vaccine for mass inoculation particularly in the control of the violent epidemic outbreaks of yellow fever in Brazil

The first samples of 17D virus arrived in Brazil from New York in January 1937 and research with this type of virus began one month later In March of the same year production of the vaccine was begun in the laboratories of the National Yellow Fever Service The vaccine was first tried out on a small number of laboratory staff but was subsequently tested on a large scale in the southern part of the State of Minas Gerais [4]

Vaccination of 59 000 persons showed (*a*) that mild reactions occurred five to eight days after vaccination in 10 / 15 of the persons vaccinated with more intense reactions in only 1 / 2 and (*b*) that the vaccine was harmless even for children and for women at any stage of pregnancy

Laboratory studies indicated that the protective antibody generally appears in the blood towards the fourteenth day after vaccination The results of protection tests carried out with a group of 882 vaccinated persons showed that about 95 of them had acquired immunity

Research carried out in the field showed that the protective action of the vaccine begins a week after inoculation even though laboratory tests may not yet reveal the presence of antibody Although the vaccine was employed at that time in areas where there was no doubt of the presence of jungle yellow fever only eight cases of the disease were observed all in persons thought to have been infected before vaccination [6]

Observations of less importance were also made on the behaviour of persons vaccinated in large numbers making it possible in 1938 to vaccinate more than a million persons with 17D vaccine and to formulate the general principle of the techniques for preparing vaccines in the field and of the in vaccination methods as described in the following pages

Organization of Yellow Fever Service

The Vaccination Section of the National Yellow Fever Service used to have a number of area vaccination organizations (vaccination sec tors) directly answerable to it each of them operating in a specific area of the country These sectors were made up of vaccination units varying in number according to the extent and population density of the areas These sectors were essentially mobile travelling either in the same direction as the epidemic waves they were called upon to combat or in the opposite direction or to regions where yellow fever was expected to appear They were principally responsible for the technical and administrative guidance of the work of the units and were directed in all cases by an experienced physician

Today the work of the Vaccination Section has been integrated with the routine work of the sector offices of the National Yellow Fever Service (SNFA) and is carried out by the same doctors who are responsible for the anti *aegypti* activities and the viscerotomy service

As far as possible the vaccination work is carried out within the geographical limits of the county (municipio) which is the basic political and administrative unit of the country

In normal times an effort is made to immunize the majority of the population directly exposed to the risk of infection particularly in the rural areas

In Brazil there are both fixed and mobile vaccination units The fixed units are of two kinds (*a*) those in the State capitals dealing mainly with persons going abroad who must have themselves vaccinated so as to obtain the vaccination certificates required by certain countries and (*b*) those established at strategic points (barreiras) in the interior of the country and which are responsible for vaccinating the masses of migrants moving

chiefly from the north east towards the south The importance of this work should be stressed for since 1934 there has been no yellow fever in the north east of the country and all persons born there since that date are therefore susceptible to the disease Moreover these are precisely the people who because of economic difficulties brought about by the periodic droughts in the north-east leave their homes in search of better living conditions and descend in continuous waves towards the south of the country known to be a yellow fever epizootic zone or—what is just as dangerous—towards the Amazon region where enzootic yellow fever is the most widespread It is our estimate that 70% of these persons enter the infected regions duly protected by the vaccine administered to them in the vaccination posts In the ports there are also units for the vaccination on board of immigrants arriving by sea

The mobile vaccination units are responsible for the most important part of jungle yellow fever prophylaxis—the routine vaccination in the counties which are considered adequately protected only when 70% of their population has been vaccinated

The sector officers are responsible for the planning and operation of programmes in the States but the heads of the vaccination units are responsible for carrying out the work in the counties included in those programmes

The heads of the vaccination units get in touch with the prefects and the local health authorities in order to interest them in the immunization campaign which they propose to carry out in the county and to obtain their support With the assistance of persons familiar with the region—usually secretaries of prefectures—and using maps of the counties they then prepare the itinerary which includes the chief towns of the districts as well as all the villages

Large plantations and farms and other places where more than 50 people may be concentrated are also included where possible in such a way that people to be vaccinated will not have to travel more than six kilometres to the immunization centres

There are places in which it is necessary to repeat the vaccination campaign in order to obtain adequate coverage of the population While there is no doubt of the great value of publicity provided by the parish priest and the school teacher and by the radio the press leaflets etc the very best publicity of all is that given by the vaccinated persons themselves Thus it is not unusual for a greater number of persons to be vaccinated in a second campaign than were reached in the first

Well planned itineraries based on a perfect knowledge of the roads and means of communications frequently make it possible to carry out vaccinations in two or three different places in one day thus saving both

time and work as well as making the vaccine more readily available to the people

Experience has shown that it is not essential that a physician be in charge of each vaccination unit Specially trained lay inspectors can carry out a rigorous supervision of all work in the field including the vaccination itself the organization of the itineraries the propaganda etc

In general the units consist of trustworthy men selected from the personnel of the anti *aegypti* service and known as guardas assisted by trained drivers These units are provided with Jeeps in order to enable them to reach the more remote parts of the counties they have to visit Certain particularly suitable drivers can be trained as assistants and even in some cases as vaccinators

The violent outbreaks of the disease occurring in 1950 1951 1952 and 1953 in the southern parts of the country made clear the advantages of each sector having its own group of vaccinators During those four years of intensive campaign against epidemic outbreaks the 120 vaccination units of the States of Mato Grosso Goiás Minas Gerais Sao Paulo Paraná Santa Catarina and Rio Grande do Sul were able to vaccinate about twelve million people

All the technical and administrative instructions are contained in a 92 page Vaccination Manual the fifth revised edition of which was published in 1946 This manual is one of eight volumes by means of which the routine activities of the National Yellow Fever Service are standardized throughout Brazil

We believe that apart from a few minor modifications of a technical nature the methods described as well as the general organization of the vaccination work are the same as those adopted by the other Latin American republics for the use of 17D yellow fever vaccine

Preparation of Vaccine in the Field

Each vaccination unit consisting of a vaccinator and an assistant — although one man can perform the work in exceptional circumstances— is provided with certain standard equipment as listed by H A Penna on page 91 In the field they prepare the vaccine in two stages first the sterilization of the equipment and then the actual rehydration The viability of the virus also used to be tested in the field by inoculating mice intracerebrally with the last portion of each preparation and a large quantity of specialized equipment had to be carried to the place of work this practice, however has been discontinued for some time being considered superfluous

Sterilization of equipment

The equipment is sterilized by boiling for a period which should never be less than 20 minutes To ensure maximum speed and efficiency the following points are observed whenever possible

(*a*) Two sterilizers are used one for sterilization proper and the other for storing and protecting the sterilized equipment to be used cool for vaccination

(*b*) If possible the equipment is replaced in the sterilizer after use in the following order so as to facilitate its removal in the reverse order needle rack covers needle racks with used needles syringes tubes small tubes empty ampoules and long needles

(*c*) The trays containing the sterilized material are flamed as are the tips of the forceps

(*d*) Thorough general sterility is maintained particularly since the vaccine itself is an excellent culture medium for micro organisms and each preparation will be used to inoculate hundreds of people

(*e*) The boiled water is changed from time to time

Rehydration of vaccine

Rehydration is carried out after all the sterilized equipment has been placed on the tray and allowed to cool completely since the vaccine is extremely thermolabile

At present the Instituto Oswaldo Cruz in Rio de Janeiro prepares only vaccine ready for dilution at 1 100 in the field and each tube contains only 1 0 cm³ of dry vaccine Consequently the first rehydration is to a dilution of 1 10 To do this 10 ml of physiological saline are introduced into an ampoule of vaccine with a sterilized syringe fitted with a long needle the saline and vaccine being thoroughly mixed in the actual tubes by means of the syringe When perfectly rehydrated the vaccine forms a homogeneous solution and the ampoule containing it is kept in ice water in a special container it is always used within three hours

Final dilution of the vaccine ready for inoculation is done with 10-ml syringes Each is charged with 9 0 ml of physiological saline and then 1 0 ml of previously rehydrated vaccine is drawn in from an ampoule giving a final dilution of 1 100 Thus the material in each ampoule is sufficient for charging 10 syringes each holding 10 0 ml The volume administered to each person is 0 5 ml so that the dried material in each tube on dilution to 1 100 is sufficient to vaccinate 200 persons

Batches from different series are never mixed

Effect of 17D Vaccine

1 *Can mass vaccination halt an outbreak of jungle yellow fever once begun?*

The reply must be in the negative or at any rate it must be admitted that it has not been possible to halt such an outbreak by this means for many reasons the chief being the following

(*a*) the speed with which the disease spreads

(*b*) the high morbidity rate

(*c*) the impossibility of obtaining immediate protection with the vaccine even if the number of susceptible subjects is small and

(*d*) the fact that with jungle yellow fever vaccination does not break the chain of infection

With jungle yellow fever the monkey and not man constitutes the reservoir of virus consequently vaccination of susceptible persons does not prevent the movement of the virus from one region to another since transmission occurs from animal to animal To prevent the appearance of human cases it would be necessary to vaccinate the entire population exposed to the risk of infection this is not feasible in practice in Brazil because of the conditions prevailing in the regions where outbreaks of the disease occur

As to the first point the reason for the speed of spread is not known The disease has been observed to break out in places which are very far from one another under conditions which suggest that the foci are interdependent rather than independent Since there is no sound basis on which to forecast the course followed by the epidemic wave it is practically impossible to organize efficient and adequate protection of rural populations at short notice

Mention should be made here of the information available on the high morbidity rate of the disease The most striking fact coming to our notice was recorded in 1950 at Colonia de Ceres in the State of Goiás where 29 000 persons out of a population of 30 000 were vaccinated starting on the day following the first fatal case of yellow fever in the locality Subsequently two other cases both fatal were recorded in the group of some thousand persons who had not been vaccinated Other more or less similar cases have been confirmed in other States where the persons attacked by the disease formed part of small non vaccinated groups—less than

When clinical cases cannot be fully investigated the morbidity rate can be calculated by assuming a case fatality rate of 10%

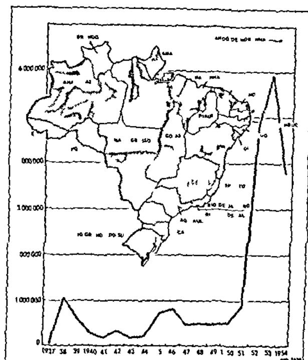
30/ of the total population Moreover careful analysis of the statistics available makes it clear that greater protection of the rural population is necessary if the occurrence of jungle yellow fever is to be completely prevented during epidemic periods in regions which are without satisfactory protection in normal times

TABLE I NUMBER OF PERSONS VACCINATED AGAINST YELLOW FEVER IN BRAZIL 1937 54

St t or co ntry of origin	Co ti s ff cted			Number of pe so s vacc at d		
	1937 49	1950 54	tot l	1937-49	1950-54	total
B a il						
Amazo as	14	11	16	157 479	98 488	255 967
Ac	7	5	7	26 968	30 759	57 727
R o B anc	—	—	—	—	—	—
G aporé	2	—	2	8 313	—	8 313
Pará	30	12	35	310 795	101 069	411 864
Amapá	3	1	3	5 627	5 971	11 598
M anhão	3	10	12	1 743	63 213	64 956
Pi I	1	2	2	1 792	19 007	20 799
C á	*1*	*14*	*14*	*10 744*	*197 154*	*207 898*
R o G a de do No te	1	15	15	15 368	157 222	172 590
Paraíba	3	4	6	9 632	52 557	62 189
Pe namb co	6	25	27	39,347	565 424	604 771
Al goas	1	5	5	2 152	64 366	66 518
Fe do do Noronha	1	1	1	2 483	84	2 567
Sergip	1	42	42	2 872	301 083	303 955
Bahia	12	43	43	125 709	946 617	1 072 326
Espí ito S nto	31	35	35	565 205	492 631	1 057 836
R o d J ei o	38	58	58	242 163	1 116,367	1 358 530
Federal D t ict	1	1	1	313 136	79 420	392 556
Mi as Ger is	163	389	390	1 340 449	3 774,211	5 114 660
Goiás	34	61	61	260 488	477,256	737 744
M to G o	20	32	34	99 154	262 968	362 122
Sã Paulo	188	208	306	1 893 041	3 073 109	4 966 150
Pa á	15	119	119	52 927	1 733 578	1 786 505
Sa ta C ta i a	19	50	50	120 315	925 474	1 045 789
Ri Grand do S l	33	61	66	170 585	1 345 153	1 515 738
Total for B il	6 8	1 204	1,350	5 778 487	15 883 181	21 661 668
A ge ti a	—	—	—	35 313	—	35 313
Boli ia	—	—	—	4,318	—	4 318
P ag ay	—	—	—	4 236	1 099	5 335
P u	—	—	—	125	—	125
G a d total	6 8	1 204	1 350	5 822 479	15 884 80	21 706 759

During epidemic periods the number of persons vaccinated in certain counties frequently exceeds the census or resident population This is due to the presence of migrants and often results in the figures for persons vaccinated apparently exceeding 100 of the county population It is

FIG 1 YELLOW FEVER VACCINATION IN BRAZIL 1937 54



Total number of persons vaccinated ag inst yellow fever 1937 54
1 706 7 9

☐ – Areas in which yellow fever vaccinat on has been carried out

—— = Numbe of persons va c nated against yellow f er

thus impossible to calculate with any accuracy the protection afforded to the inhabitants of such places Moreover it should not be forgotten that these vaccination figures include revaccinated persons and that the aggregate population figures include children und r one year of age most of whom have not been vaccinated they represent an appreciable p rcentage of the rural population Other groups which must not be overlooked are persons who, through negligence or lack of interest have not had themselves vaccinated and sick persons who are unable to travel Table I and fig 1 3 show the extent of yellow fever vaccination in Brazil over the period 1937 54 and more particularly over the period 1950-54

Observation of several outbreaks has shown that there is definite correlation between the decline of an epidemic and the decrease in the number of mosquitos in the forest areas The number of human cases

FIG 2 AREAS OF BRAZIL IN WHICH CASES OF YELLOW FEVER WERE RECORDED IN 1950-54



Altogethe 323 cases of j gle y llow fe r were co fi m d in 118 c u

of jungle yellow fever decreases with the lowered density of infected mosquitos in the forests following the end of the rains and the rainfall curves are parallel to those for yellow fever cases

The foregoing in no way detracts from the protective value of vaccination but it must be repeated that vaccination can only be completely effective in an epidemic period when it is applied to the entire population at risk and this is generally a practical impossibility

Studies carried out in São Paulo clearly show that this extreme protection is not economical even in counties whose geographical situation and vegetation particularly favour the occurrence of future epizootic outbreaks of yellow fever Vaccination of 70 of the population offers a good security margin particularly when it covers mostly a rural population

FIG 3 AREAS OF BRAZIL IN WHICH YELLOW FEVER VACCINATIONS WERE CARRIED OUT IN 1950-54



Altog ther 15 884 280 persons were vaccinated aga nst yellow fever in 1,204 counties

2 *Does 17D vaccination give immediate protection against yellow fever infection?*

It has already been pointed out that judging from experiments in the field the protective effect of the vaccine begins towards the seventh day after inoculation [3 5 6] although the presence of antibodies in the blood at this time cannot be demonstrated by the present laboratory methods Furthermore laboratory experiments [5 6] indicate that seven days after 17D vaccination monkeys have acquired a strong immunity against virulent pantropic virus although antibody may not be demonstrable in their blood

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See al o the contr bution by G Courtois page 105

Only very rare cases are known in which the disease has occurred in human subjects shortly after vaccination and in these cases it may be supposed that the persons concerned were already infected at the time of vaccination On the other hand it must be remembered that in view of the huge numbers of persons vaccinated over very wide areas of the country it may well be that cases of yellow fever have occurred after vaccination of which nothing is known It should be pointed out however that the public nowadays has full confidence in yellow fever vaccine and that such cases would almost certainly be immediately brought to the notice of the health authorities This happened in 1939 in the southern part of the State of Espirito Santo where batches with little or no antigenic potency were inadvertently used with the result that cases of yellow fever occurred among recently vaccinated persons

It may therefore be supposed that a relative degree of protection is established within a short time of vaccination with 17D vaccine This view is supported by observations made in the field

3 *Is 17D vaccine harmless?* [b]

An affirmative answer is now given to this question although cases of jaundice and encephalitis have been recorded in the past the first of them in Brazil being observed in 1940 In August of that year the practice of adding 10% normal human serum to the vaccine was given up and cases of this type ceased to be observed The practice had resulted in the trans mission of the virus of infectious hepatitis which for many years conta minated yellow fever vaccine At the time a very thorough study of the problem was made in the State of Espirito Santo where it was found that the average incidence of the disease among persons vaccinated with ictero genic batches was 5 % The average incubation period of the disease was four months and the symptoms were very similar to those of what used to be called catarrhal jaundice The severity of the disease covered the whole range from mild to extremely serious and there were some fatal cases

Cases of encephalitis attributed to an unusual increase in the virulence of the virus itself were also reported in 1941 These cases occurred in only 0 5% of vaccinated persons and the incubation period was variable ranging from 8 to 15 days The disease was accompanied by severe nervous symptoms lasting on the average 11 days but there were no deaths

It should be noted that not a single case of jaundice or encephalitis has been observed in Brazil since 1940 and 1941 respectively and that although living virus is used in yellow fever vaccine the possibility of any kind of accident must be considered extremely remote

[b] See also the co trib t on by G St a t p ge 143

Statistical Data and Reporting

The data relating to vaccinated persons—name address age sex colour urban or rural domicile initial vaccination or revaccination—were formerly entered in special registers but this practice was given up when the number exceeded one million Today the identity slips filled out at the time of vaccination (see Annex 1) are grouped by preparation and kept on file in the sector offices for a period of not more than one year after which they are destroyed For each preparation of vaccine in the field the special form shown in Annex 2 is filled out and attached to the identity slip of the last person to receive that preparation alternatively a stamp may be used to enter the same information on the back of the last slip

The units send a weekly summary form (shown in Annex 3) to the sector office listing all the preparations made during the period the dates of vaccination places at which vaccination was done number of persons vaccinated the batch numbers of vaccine used the quantities (ml) inoculated and the dilution given as well as a statement of the equipment available to the unit for work in the coming week The sector office in turn compiles a weekly report to the central office (Annex 4) grouping together on a special form all the data of interest contained in the weekly summaries submitted by the units for which it is responsible

It will be clear from the foregoing that the administrative part of vaccination work in the field as well as in the office has been reduced to a minimum The statistical data collected are restricted to those essential for guiding and following up vaccination in the field

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ANNEX 1

VACCINATION IDENTITY SLIP (FORM FA 80)

NATIONAL YELLOW FEVER SERVICE

(Please write clearly)

Name

Address

Urban ? Rural ?

Age Sex Colour

Revaccinated against yellow fever ?

ANNEX 2

VACCINE PREPARATION RECORD

NATIONAL YELLOW FEVER SERVICE

VACCINE PREPARATION RECORD

State

County

District

Place

Date Preparation No

Time Last inoculation

Batch No Quantity (ml)

Dilution Total vaccinated

No of days without ice

Signature

ANNEX 3

WEEKLY SUMMARY OF VACCINATIONS SUBMITTED BY UNITS

NATIONAL YELLOW FEVER SERVICE

WEEKLY SUMMARY OF VACCINATIONS

1st week of 1954

State Minais Gerais County Miraí From 3/1/54 to 9/1/54

(Use e l e f a h p p r t o)

Local ty	Date	Number of pe s ns acc n t d			Vac e		
		u b	r (	t t (	batch n	q a t ty (ml)	d ton
Dori d Vito i	3/1		Sund y				
D ri d Vit ri	4/1	–	173	173	244	0 5	1:100
Do is d Vito ia	4/1	–	195	195	244	0 5	1 100
Do ia d Vito ia	4/1	–	78	78	244	0 5	1:100
S Seb sti da Vi ta Al g e	4/1	–	197	197	244	0 5	1 100
S Seb ti d Vista Al gre	4/1	–	117	117	244	0 5	1 100
S S b sti d Vi t Al g	4/1	–	19	19	244	0 5	1 100
Ch E t t	5/1	–	107	107	244	0 5	1 100
ão Jo e Est t	5/1	–	195	19	244	0 5	1 100
Sã Jo E t t	5/1	–	53	53	244	0 5	1 100
	6/1		S t	asid	r p	opagand	
Varg Al g Est t	7/1	–	200	200	244	0 5	1 100
Varg Al gr E t ta	7/1	–	100	100	244	0 5	1 100
São Vic nt Est t	8/1	–	149	149	244	0 5	1 100
Sã Vic t Est te	8/1	–	61	61	244	0 5	1 100
P t imo io	9/1	–	200	200	244	0 5	1 100
P trimoni	9/1	–	200	200	244	0 5	1 100
P t imoni	9/1	–	50	50	244	0 5	1 100
			W k in	hi	omu e	nded	n 9/1/54
TOTAL	–	–	2 094	2 094	–	–	–

Stocks held by u t		Va um fla ks		Tot l mb f pe ns c at d	
Doses of vaccine	9 200	1 gallon	2	Urban	–
Ampoules of serum	400	3 gallons	0	Rural	2 094
FA-80 form	19 000	5 gallons	0	Grand total	2 094

S g at e of v cinato

ANNEX 4

WEEKLY REPORT OF YELLOW FEVER VACCINATIONS

NATIONAL YELLOW FEVER SERVICE

WEEKLY REPORT OF YELLOW FEVER VACCINATIONS

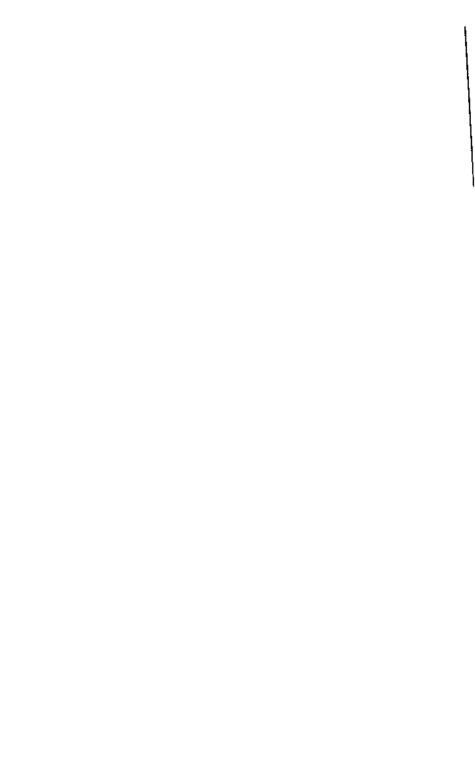
Reg on South   Ar a Ri G and d Sul   State Ri G and d S l   48th Week From 28/11 to 4/12/54

U H dq rt rs	U t pers (ac t)	U t pers (t t)	U t pers (d er)	U t pers (t tal)	U t k (d t)	U t k (mp f)	U t k m fl k (g ll) 1	U t k m fl k (g ll) 3	U t k m fl k (g ll) 5	V h l (typ)	V h l (SFA N)	V d (d)	V d (f t (b k amp))	N mb f p t d (be)	N mb f p t d (l)	N mb f p t d (t t l)
B te G gal	1	–		1	4 800	330	1					532	200	672	2 396	3 068
En an ad	1			1		220	1				–	343	200		2 259	2 259
Farroupilh	1			1		440	1					120	–	2 125	2 355	4 480
Garibaldi	1		1	2	2 800	450	1			J p	473	890	800		2 720	2 720
Laj ad	1		–	1	3 600	570	1	–				510		–	490	490
No te g	1			1	1 000	505	1					530			670	670
P to Al gre		–			200	131						195		5	–	5
San Mari	1			1	600	6 613	16	1		J p Je p	439 432		400			–
Sant R	1	–		1	5 000	750	1			J p	477	653	–		947	947
São J rônimo	1			1		100	1			Je p	449	377		–	3 423	3 443
Taquarí	1			1	4 000	396	1					179	200		421	421
Vacari	2		–	2	5 400	435	1		–	J p	455	514	–	–	1 086	1 086
V ardpoli	1		–	1	600	426	1			J p	459	510	200	–	2 890	2 890
TOTAL	13	–	1	14	28 000	11 346	27	1	–	?		5 351	2 000	2 802	19 647	22 449

C mulative total of perso s acci ated du g yea   Urba 69 1 1   Rur l 342 406   Total 411 537

Sp t re

POST VACCINATION REACTIONS



REACTIONS FOLLOWING VACCINATION AGAINST YELLOW FEVER

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I. INTRODUCTION

Development of Immunizing Strains of Yellow Fever Virus

Until 1930 efforts at securing active immunization against yellow fever were directed largely towards the inactivation by chemical or physical means of the virus intended for use as vaccine. All such efforts proved unsuccessful, no chemical or physical method having been found capable of *eliminating the pathogenic and at the same time retaining the antigenic* properties of the virus.

In consequence attention was concentrated on discovering a living virus adaptable to human use, and in the years subsequent to 1930 several methods of immunization with such a modified virus as the antigenic element were devised.

French neurotropic virus

Of these methods involving the use of active modified virus, the first was based on the work of Theiler, who, in his search for susceptible laboratory animals additional to the monkey, had discovered that the white mouse was receptive to an intracerebral but not to an intraperitoneal inoculation with the French virulent pantropic strain of virus—a strain previously shown by Mathis, Sellards & Laigret[44] to have been transmissible and infective to rhesus monkeys by the injection of these animals with blood taken early in the disease from a human case of yellow fever at Dakar. Moreover, prolonged serial brain to brain passage in mice was found by Theiler[73] to have exerted so profound a modification in the behaviour of the original pantropic strain that, while on the one hand it showed an increased neurotropism for mice and, as first reported by

Sellards[57] *for monkeys*—a *result* evidenced by its producing in these animals when injected intracerebrally a rapidly developing fatal encephalitis—it had on the other hand lost its viscerotropism for monkeys i e its ability to produce visceral yellow fever *in* these animals when injected extraneurally Monkeys injected extraneurally with this modified strain developed as a rule only a mild non fatal systemic infection (but see below) and later possessed an active solid immunity to unmodified highly virulent strains of yellow fever virus

This mouse brain virus known as the French neurotropic yellow fever virus was although possessing an enhanced neurotropism considered to be capable of use in human vaccination because of the marked diminution of its viscerotropic affinity and was therefore employed at its 105th to 176th passage level as the antigenic element in the first vaccine ever used to immunize man against yellow fever that prepared by Sawyer Kitchen & Lloyd [52, 53] *Because however in the view of these workers the increased* neurotropism of this virus rendered it potentially dangerous for human vaccination they employed *in addition to the virus vaccine* sufficient specific immune serum to prevent the virus from entering the circulating blood of the inoculated person thus affording protection against any pathogenic activity present in the virus Immunization by this means was effected by a single injection of a mixture of dried living virus fixed for mice and of human immune serum with separate injections of enough additional immune serum to make up the amount required for protection—the amount of virus and immune serum being originally 0 003 g and 0 3 ml per kg of body weight respectively

The view expressed above that the increased neurotropism of the mouse adapted virus rendered it potentially dangerous for human vaccination unless accompanied by adequate amounts of specific immune serum was soon endorsed by Findlay [13] and by Theiler & Whitman [7] Moreover support to this view was shortly lent by the results of experimental work which showed that a significant percentage of rhesus monkeys inoculated extraneurally with the virus—subcutaneously intraperitoneally by scarification of the skin (Theiler & Hughes [7]) or by nasal instillation (Findlay & Clarke [14])—develops fatal encephalitis In this connexion Theiler & Smith [5] reported that among monkeys after subcutaneous or intraperitoneal inoculation or the application of the virus to the scarified skin fatal encephalitis had been observed in approximately 50% of cases Thus of 21 monkeys inoculated extraneurally eight died from this cause four among 10 inoculated subcutaneously with the virus at its 119th mouse passage (3) or at its 293rd (1) two among eight inoculated intraperitoneally with the virus at its 227th mouse passage and two among three to which the virus at its 293rd passage had been applied by scarification of the skin

In the experience of Theiler & Whitman [77] such a result occurred particularly among younger animals or animals receiving either small doses of virus alone or inadequate simultaneous inocula of protecting immune serum These authors emphasized the potential hazards attendant on the use of neurotropic virus alone particularly for children

Nevertheless the immunization of human beings by the administration of this same neurotropic virus unaccompanied by specific immune serum has been continuously and extensively practised mostly by French workers since 1932

The first method of immunization by the use of the mouse adapted virus alone was that proposed by Sellards & Laigret [58] it consisted in the inoculation of the virus at its 134th serial passage in mouse brain each person inoculated receiving 1 ml subcutaneously of a dilution (1 1 000 1 10 000 or even 1 100) of the infected brain in normal saline solution containing 10/ rabbit serum The occurrence of a number of untoward reactions following attempted immunization by this means led Laigret [29, 30] to prepare a second type of vaccine consisting of the mouse brain virus attenuated by exposure in glycerol to a temperature of 20 C and dried in the presence of sodium phosphate Three injections of virus exposed to this temperature for four days two days and one day respectively were given at 20 day intervals In order to reduce the number of injections and thereby to make immunization more widely applicable Nicolle & Laigret [45] introduced a single dose method of vaccination employing mouse brain virus which after one day s exposure in glycerol to 20 C and subsequent desiccation was with the object of retarding the diffusion of the material from the site of inoculation coated with a layer of egg yolk or of olive oil or with a double envelope of both these agents Later Nicolle & Laigret [46] stated that the m thod selected for immunization in French West Africa Cameroons and the Lake Chad Region was a single inoculation of this dried virus coated simply with egg yolk

As regards the method of attenuation of the virus described by Laigret [30] and referred to above according to Laigret drying at 20 C for one day resulted in a reduction of the virus content from for example an original 32 000 mouse units to 3 200 a progressive and proportionate reduction occurred after further exposure until after four days drying there remained only traces of virus insufficient to kill mice [29, 30] It was shown by Theiler & Whitman [77] however that treatment of the virus so effected resulted not in attenuation but only in a progressive decline in the amount of living fully virulent virus present in the preparation and that the method of immunization consisted therefore in the administration of graded doses of fully virulent neurotropic virus

The Laigret mouse adapted virus prepared from the French strain at its 130th to 185th mouse passage by one or other of the two last mentioned

methods was however the first to be used on a wide scale Thus in French West Africa between June 1934 and December 1935 the three injection method was used on 9 592 persons of whom 5 699 received the full immunizing course of three injections

But subcutaneous inoculation with such virus did not for various reasons adequately meet the requirements of the French authorities who confronted with the problem of yellow fever control in their vast territories in tropical Africa recognized as of prime importance the need for mass immunization of the indigenous populations then so much at risk Search for a vaccine which would be at once safe effective easily administered and inexpensive was therefore pursued particularly at the Institut Pasteur Dakar and finally resulted in the development by Peltier et al [49, 50] of a preparation of the neurotropic yellow fever virus at its 238th passage through mouse brains which could be applied to the skin by mild scarification thereby replacing subcutaneous inoculation and overcoming such major difficulties inherent in mass immunization programmes as the provision in adequate number of syringes and needles thoroughly sterilized Since 1940 this scratch or topical method of immunization has been adopted for use mainly by the French authorities and particularly in French tropical Africa where some 56 million vaccinations have been so performed

The technique of preparing and administering the vaccine in question was fully described by Peltier [47] in 1946 when it was stated that the vaccine was made from the brains of mice inoculated with the French strain of virus at its 256th to 258th passage in mice There can be no doubt about the high immunizing property possessed by this mouse brain vaccine applied by scarification either alone or as is commonly practised in French tropical Africa in combination with smallpox vaccine Moreover it was felt that by the use in this new Dakar vaccine of the virus at its 256th to 258th passage there might be avoided those untoward reactions which on occasion had been observed to follow the inoculation of former vaccines containing the neurotropic virus when at a lower passage level and when probably not so fixed as it had become with later animal passage

Asibi virus derivatives

17E Reference has been made earlier in the text however to the view held by several workers that the increased neurotropism for mice and monkeys of the mouse adapted French strain rendered it potentially dangerous for human immunization Because of this view a search was made to discover further methods of modifying yellow fever virus—methods which would not only reduce its viscerotropism but would also diminish or at least not augment its neurotropism The desired modification was

eventually achieved by the prolonged cultivation in tissue in vitro by Lloyd Theiler & Ricci[87] and by Theiler & Smith [4, 5] of the highly virulent pantropic Asibi strain—a strain originally derived from and named after an African suffering from yellow fever at Kpeve Gold Coast and transmitted by the inoculation of the blood of that patient to a rhesus monkey on 30 June 1927 by Mahaffy and Bauer The series of cultures with the Asibi strain which was established by Lloyd Theiler & Ricci[22] represented the 17th set of tissue culture experiments by these workers and in order to distinguish between the various series of cultures Theiler & Smith[24] added initials to the experiment numbers The Asibi strain was first established and maintained in tissue culture containing minced mouse embryonic tissue plus 10% normal monkey serum in Tyrode s solution and the series cultivated in this medium was designated 17E This variant 17E was found to have lost to a great extent its viscerotropic affinity for monkeys without acquiring increased neurotropism for mice and as a result was substituted by Lloyd[20] for the mouse brain virus in the serum virus method of human vaccination The reduction of viscerotropism in the cultured virus was not however considered sufficient to warrant its use without the protection of immune serum the virus was still capable of producing in monkeys a fairly severe systemic infection Lloyd s method consisted in the inoculation of known quantities (between 25 000 and 170 000 minimum lethal doses (MLD) for mice) of the virus at its 130th passage in mouse embryonic tissue together with amounts of specific immune serum ranging from 0 5 ml to 0 6 ml per kg of body weight—previous experience having shown that such amounts were sufficient to prevent the circulation of virus in the peripheral blood This method was efficient but in common with other serum virus methods was cumbersome impracticable for field application and not adapted to mass vaccination

17D Further cultivation of the Asibi virus by Lloyd and his collaborators using successively minced mouse-embryo with Tyrode s solution medium (18 passages) whole chick-embryo with Tyrode s solution (58 generations) and thereafter a medium in which the tissue component was minced chick embryo from which the brain and spinal cord had been removed before mincing then led to the development of the branch known as 17D—a variant which showed not only a loss of neurotropism for mice and monkeys alike but also a markedly diminished viscerotropism for the latter animals Thus in mice although the strain could still produce encephalitis it could do so only after a somewhat increased incubation period in monkeys it had altogether lost its ability when inoculated intracerebrally to produce fatal encephalitis Monkeys inoculated extraneurally with this 17D virus had no fever or other signs of illness their blood contained but minimal amounts of virus they were shown to have

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developed specific antibodies and were solidly immune to highly virulent pantropic strains The loss of both viscerotropic and neurotropic affinities as demonstrated in monkeys made this variant in the opinion of American and English workers the virus of choice for human vaccination it also being free from the risk of extraneous infections from latent viruses of mice which might be pathogenic for man As regards the manufacture of 17D vaccine inasmuch as the titre of virus produced in tissue culture is low vaccine is prepared from developing chick embryos inoculated with culture virus—fresh fertile hens eggs after seven to nine days incubation being inoculated with 0 05 ml of the 200th to 300th subpassage material The vaccine is and has been since 1942 of the aqueous base (serum free) type the technique of its production is described by Hargett Burruss & Donovan [21]

For human immunization 17D vaccine has since 1937 been administered by subcutaneous inoculation and to this end some 50 million doses have been distributed

With a view however to developing a vaccine which would be not only safe and efficient but also comparable with the Dakar vaccine in its suitability for mass vaccination and in its low cost of production and application investigations into the possibility of employing 17D virus vaccine by scratch were commenced in 1947 at the Yellow Fever Research Institute in Lagos Nigeria Hahn [22] described the mode of preparation of the vaccine and the results obtained from its use The vaccine produced by grinding the whole virus infected chick-embryos with gum arabic solution and desiccating the homogenized mixture to powder form proved when reconstituted in sterile distilled water at the time of use easy of

TABLE I DIFFERENCES IN PATHOGENICITY BETWEEN FRENCH NEUROTROPIC VIRUS AND 17D VIRUS

Virus	Mice	Rhesus monkeys	
	average time of death after intracerebral inoculation (days)	intracerebral inoculation	extraneural inoculation
French neurotropic	4–10	fatal encephalitis	fever in approximately 5[illegible] of animals virus present in circulating blood for [illegible]5 days fatal encephalitis in approximately 30[illegible] of animals
17D tissue culture	8–[illegible]0	non fatal encephalitis	occasional fever minimal amount of virus in circulation no deaths

Based on Theiler & Smith Table V

administration by the scratch technique From results obtained both in the laboratory and in a field trial at Kumba Fiango British Cameroons where 3 808 of the inhabitants were vaccinated by this method Hahn concluded that the 17D strain of yellow fever virus could be applied by scratch with the production of a level of immunity of the same order as that resulting from subcutaneous inoculation of the virus Further evidence in this sense was adduced by Horgan at the Virus Research Institute Entebbe[9, 10] and by Dick[7] in respect of groups of individuals so immunized in Uganda

To summarize the foregoing there are in current use for the immunization of man against yellow fever the French neurotropic virus and the 17D virus the main differences between these have been shown by Theiler & Smith[75] and are reproduced in table I

Methods of Immunization Involving Use of French Neurotropic Virus and 17E and 17D Viruses

The methods of immunization devised since 1930 may be summarized thus

A Using French neurotropic virus

(1) By inoculation together with immune serum

(*a*) Sawyer Kitchen & Lloyd[5, 53] (1931 1932)

(*b*) Aragão[1, 2] (1933) preliminary intramuscular injection of 3 ml of human immune serum into deltoid followed 6 hours later by 2 ml of immune serum + 0 001 g of fresh virulent brain of camondongo (Brazilian house rat)

(2) By inoculation without immune serum

(*a*) Sellards & Laigret[68] (1932) single injection of fresh virus

(*b*) Laigret[9, 30] (1934) 3 injections at 20 day intervals of dried virus attenuated by exposure to 20°C for 4 days 2 days and 1 day respectively (Laigret s three injection method)

(*c*) Nicolle & Laigret[45] (1935) single injection of dried virus attenuated by exposure to 20°C for 1 day and incorporated in egg yolk *(Laigret s single dose method)*

(3) By scarification of the skin

(*a*) Peltier et al[49, 50] (1939 1940)

(*b*) Peltier[4] (1946)

B Using derivatives of Asibi strain

17E

(1) By inoculation together with immune serum

(*a*) Lloyd[36] (1935)

17D

(1) By inoculation without immune serum

(*a*) Theiler & Smith[5] (1937)

(*b*) Hargett Burruss & Donovan[24] (1943) vaccine of the aqueous base (serum free) type

(2) By scarification of the skin

(*a*) Hahn[23] (1951)

II POST VACCINATION REACTIONS

Reactions have in varying number type and degree of severity followed the use of all except perhaps the last mentioned (Hahn[23]) of the immunizing methods listed above According to whether or not they have involved the central nervous system and because therefore of their relative importance these reactions are dealt with here under two separate categories Their occurrence in each of these categories is considered under the several methods of immunization listed above and in the order there shown the etiology of reactions involving the central nervous system is discussed in part III (see page 174) under a separate heading immediately after the account of their occurrence while that of reactions not involving the central nervous system is included within the narrative of their occurrence

Category 1

Local and General Reactions not Involving the Central Nervous System

French neurotropic virus

(1) By inoculation together with immune serum

Sawyer Kitchen & Lloyd[53] reported three types of reaction *one on* the same day as the vaccination with slight rise of temperature (37° 38°C) tenderness redness and swelling near the sites of inoculation aching in limbs headache and backache lasting one to three days a second with onset between the 6th and 12th post vaccination days and with symptoms as above lasting two or three days considered to be due to the inoculation

of foreign tissue or to the immunizing virus itself kept in check by the immune serum and a third appearing on the 13th day after inoculation and showing symptoms of serum disease—rise of temperature swelling of the joints etc Findlay[13] described three types of reaction following the inoculation of 200 persons with varying amounts of serum and virus one within 48 hours of inoculation with rise of temperature to 102 F (38 9°C) or more frontal headache postorbital pain photophobia aching in muscles of back and shoulders and lassitude lasting for one or two days a second with similar symptoms and time of onset but after a return to normal showing an exacerbation of these symptoms plus nausea lasting for one or two days and a third in 35 (21 9 %) of 160 vaccinated persons with onset between the fourth and seventh post vaccination days and with similar symptoms plus hyperaesthesia of the skin lasting two to three days Aragão[1] noted only occasional rise of temperature with malaise on the seventh or eighth post vaccination day Stefanopoulo[68] reported among 103 persons vaccinated with virus together with horse hyperimmune serum no reaction apart from the febrile reaction following any serum therapy except in one case which was described by Darre & Mollaret[5] and is referred to under category 2 (see page 159)

(2) By inoculation without immune serum

Single injection of fresh virus Laigret[79] recorded the occurrence of jaundice haemorrhages and albuminuria in three persons who had been inoculated 13 15 days previously with fresh virus in a 1 10 000 suspension (See also under category 2 page 159)

Laigret s three injection method (attenuated virus) Mathis Laigret & Durieux[43] reported that among 3 196 Europeans vaccinated in French West Africa by this method there was no local reaction but that in a certain proportion of cases there occurred on the sixth day after inoculation a reaction characterized by moderate fever headache postorbital pain and backache lasting from 12 to 30 hours Sixth day reactions were also described by Sorel[66] who basing his observations on the results of the immunization of over 9 000 persons in French West Africa stated that such reactions typically presented as symptoms moderate fever frontal headache backache and mild joint pains together with some degree of asthenia and anorexia These reactions were ordinarily of short duration (one to two days) but in about one third of the cases symptoms including in addition nausea and vomiting were more pronounced and lasted from three to nine days In Sorel s view these general reactions classified respectively as mild and of moderate severity were of a systemic nature evidencing the viscerotropic activity of the neurotropic strain of virus used Occasionally however as early as the sixth post vaccination

day reactions indicating involvement of the central nervous system were observed (see under category 2 page 158) conversely the general reactions were frequently delayed in their appearance until the ninth day or later Martin Rouesse & Bonnefoi[39] noted only a febrile reaction about three days after vaccination

Laigret s single dose method (attenuated virus) Mathis Durieux & Mathis[41] reported that among 376 Europeans observed after vaccination at Dakar by this method 150 showed reactions of which 67 were classified as mild 49 as moderately severe and 34 as prolonged mild with onset five to six days after vaccination with headache malaise lassitude but no fever moderately severe with onset on the sixth to eighth post vaccination day with headache backache and fever (37 5° 39 5°C) up to 3 days and prolonged with onset 16 to 24 days after vaccination and with symptoms and signs of central nervous system involvement (see under category 2 page 161) Of 50 Negroes similarly vaccinated none showed any reaction Jadin & Arnaldi[25] found that among 2 257 persons—37 Europeans and 2 220 Africans—so vaccinated in Zongo Belgian Congo three different types of reaction occurred mild moderately severe and prolonged Among the 29 reactions observed among the Europeans 12 were mild with onset on the fifth to sixth day fever to 38°C and lassitude 14 moderately severe with onset on the fifth to sixth day fever to 39°C intense headache and backache necessitating stay in bed and 3 prolonged with onset on the 14th day and with symptoms of central nervous system involvement (see under category 2 page 161) All three types were also noted among the Africans

(3) By scarification of the skin

Peltier[45] summarizing the results of over 20 million vaccinations by this method in French West Africa described two types of reaction which had been observed to occur an early one in 10% 15% of those vaccinated with onset on the fifth or sixth post vaccination day characterized by fever headache and backache lasting for a day or two and a delayed one of rare occurrence 12 to 15 days after vaccination consisting of a serious meningo-encephalitic syndrome lasting five or six days The former type was held to be a systemic response to the invasion of the blood by the virus the latter to be due to an invasion of the nervous system by the virus (see under category 2 page 162) Macnamara[38] classified the reactions which followed vaccination by this method of the African population in a limited area of southern Nigeria as mild viscerotropic and neurotropic The first two types had no sharp distinguishing features between them the last type was distinct both in symptomatology and in incubation period and is considered in detail under category 2

(see page 166) Mild reactions occurred four to five days after vaccination the temperature rose sometimes reaching 102° 103°F (39° 39 5°C) within the next 24 hours thereafter it fell to normal in one or two days in some cases there was headache less often backache and abdominal discomfort were experienced symptoms sometimes persisted one or two days after the temperature had returned to normal The few viscerotropic reactions which were observed commenced several days after vaccination and showed in addition to fever headache backache and abdominal pain vomiting albuminuria and jaundice by the end of the tenth post vaccination day no abnormal signs or symptoms were noted except for slight jaundice Durieux reviewing the results of twelve years experience with this method in French West Africa (see page 47) states that the general reaction when it did occur appeared regularly on the fourth or fifth post vaccination day This reaction was characterized by fever headache and stiffness—symptoms which occurred during the period when the vaccine virus was multiplying in the blood of the vaccinated person it was ordinarily of short duration (24 hours) but might b prolonged for several days and be of greater or lesser intensity Its p rcentage of incidence varied with the age and state of health of those vaccinated being low among the young and healthy and high (up to 50) among the old and debilitated A second type of reaction delayed until after the tenth post vaccination day and of rare occurrence is also described it is considered under category 2 (see page 163)

Derivatives of the Asibi strain 17E

By inoculation together with immune serum

Lloyd [38] reporting on the inoculation of this strain in a dosage varying between 25 000 and 170 000 MLD for mice together with human immune serum in amounts of 0 5 0 6 ml per kg of body weight stated that this quantity of serum was sufficient to prevent the circulation of virus in the peripheral blood and that no reactions of any importance resulted Thus of 20 persons so immunized 13 had no reaction while only 3 had fever up to 37 7 C and 4 a rise of temperature of 1 °C or less Soper & Smith [61] reviewing in 1938 the results of vaccination in Brazil with 17E together with human immune serum with hyperimmune goat serum and with hyperimmune monkey serum severally observed that reactions occurred as follows

With 17E + human immune serum of 44 persons so inoculated 4 had headache grippe like symptoms with low fever (maximum 38 3 C) commencing on the fourth to sixth post vaccination day and some soreness and pain at the site of the serum inoculation disappearing after a few hours

With 17E + hyperimmune goat serum in a dosage equivalent to twice the antibody content of standard human immune serum reactions attributed to the serum occurred between the third and tenth post vaccination days in 176 of 207 persons inoculated local urticaria was most frequent but in 6 cases there was generalized urticaria accompanied in 3 cases by serum sickness Reactions with onset between the 4th and 17th post vaccination days mostly between the 10th and 13th and considered to be due to the virus itself were discovered in 44 persons of these 28 had headache body pains and slight fever for one or two days 13 had similar symptoms but severe enough to necessitate their staying in bed for one day or more and 3 had symptoms of short duration very suggestive of mild yellow fever These reactions were attributed to the early elimination of the heterologous serum which resulted in the disappearance of the antibody content of such serum before sufficient immunity had developed to prevent the appearance of clinical symptoms due to the virus infection

With 17E + hyperimmune monkey (rhesus) serum among 795 persons so vaccinated there was no serious reaction but there was a high percentage of failure to immunize which was attributed to the excessive amounts of antibody in the serum component resulting in a complete inhibition of the activity of the virus

Derivatives of the Asibi strain 17D

(1) By inoculation without immune serum

Theiler & Smith [5] the first to use this method reported that of 8 persons inoculated 5 had a febrile reaction (37°C) slight headache and backache but no absence from work was caused thereby In 1937 Soper [6] stated that of 5 000 persons vaccinated in Brazil 5%-8% showed on the sixth or seventh post vaccination day slight headache and sometimes febrile reactions lasting one or two days Smith Penna & Paoliello [59] in their review of 59 532 vaccinations carried out in Brazil in the 12 month period ending 31 January 1938 recorded that no local reactions occurred at the site of inoculation except slight redness in a few cases and that no evidence of allergic reaction to the chick-embryo protein was noted General reactions with onset between the fifth and eighth post vaccination days and affecting from 10%-15% of those vaccinated were mild in type ordinarily consisting of headache backache low grade fever grippe like pains and malaise and lasting from a few hours to a few days Only 69 (0 18%) of 32 000 vaccinated had reactions severe enough to cause loss of time from work Thus in one group of 2 457 persons vaccinated 360 (14 6%) had headache of from one to two days duration 250 (10 2) developed headache and body pains 34 (1 4%) missed work for a day

and 4 (0 16%) spent one day or more in bed In a few instances among the total vaccinated symptoms which might have been ascribed to the virus appeared as early as the third and as late as the tenth post vaccination day There were no delayed reactions

Fox et al [18] however reported the occurrence of reactions of three degrees of severity following routine immunization in Brazil during 1941 with a series of vaccine lots derived in particular from a single substrain of 17D virus which on later examination proved to have developed a marked increase in its neurotropism during a very small number of sub-cultures away from the parent strain Among a total of 69 843 persons vaccinated—55 073 in the Guanhães area and 14 770 in the Guaxupe area—539 uffered reactions

128 persons had mild reactions with moderate headache body aches fever often accompanied by chills and mild asthenia some loss of appetite was common nausea and vomiting were infrequent the average total duration of illness was two days

157 persons had more pronounced reactions with fever more intense headache and a greater degree and frequency of anorexia nausea vomit ing and asthenia delirium mostly in children sometimes occurred at the peak of the febrile reaction symptoms persisted for five or more days confinement to bed for two or more days was not uncommon The 285 reactions just described had their peak of occurrence six to eight days after vaccination and represented the syst mic reactions usually provoked by the 17D strain It must be mentioned however that cases with the more aggravated symptomatology continued to occur in relatively large num bers up to the 17th post vaccination day and

254 persons had serious reactions with symptoms particularly referable to the nervous system these are considered under category 2 (see page 168)

More recent reference to the reactions which may follow the sub cutaneous inoculation of 17D virus vaccine as now prepared has been made by Soper [53] and by Kerr [28] In this connexion Soper states that inoc ulation may be followed by slight symptoms of malaise five to seven days later while Kerr observes that the normal reaction to such inoculation is perhaps the mildest reaction to any known vaccine

(2) By scarification of the skin

Following the application of this method to 3 808 Africans in the British Cameroons and to 153 African volunteers during two controlled experi ments in Uganda no reactions of note were reported either by Hahn [23] or by Dick [7] respectively

Allergic reactions

This review of the literature dealing with systemic reactions to 17D vaccine would it is felt be incomplete without some reference being made to foreign protein reactions to that vaccine and to hepatitis which as will be seen below at one time complicated the use not only of 17D vaccine but also of certain other yellow fever vaccines

Allergic reactions to 17D vaccine have apparently been very infrequent despite the fact that the vaccine consists of the tissue juices of chick embryos *Such as have occurred have ordinarily been mild developing* a few hours after inoculation and consisting of urticaria or of vague malaise lasting from a few hours to a day or two More serious reactions of this nature have however been reported Thus Sulzberger & Asher [70] reported three cases of urticarial and erythema multiform like skin eruptions in men of the United States Navy who had received injections of different lots of the vaccine Swartz [71] published the record of a man who developed a violent allergic reaction—angioneurotic oedema marked urticaria gastro intestinal symptoms and severe dyspnoea—a few minutes after receiving a single injection of cholera vaccine and one of yellow fever vaccine on subsequent investigation this man was found to be extremely sensitive to egg and chicken muscle proteins Sprague & Barnard [6] described the case of a man who within 15 minutes after receiving his inoculation developed severe asthma oedema of the face and generalized urticaria this man gave a history of eczema and asthma and of long standing sensitivity to white of egg More recently in connexion with this subject it has been stated by Kouwenaar [26] that the risk of allergic reaction following yellow fever vaccination particularly in allergic individuals is by no means inconsiderable Thus among 1 130 persons inoculated with aqueous base 17D vaccine 4 showed a recrudescence of a pre existing *allergy (asthma hay fever or eczema) early urticaria was seen in 2 allergic* persons and late reactions resembling serum sickness were observed in 9 individuals most of whom had a history of allergy In Kouwenaar's view this risk will be obviated only when the scarification technique is substituted for subcutaneous injection

Hepatitis

The occurrence of acute hepatitis as a complication of yellow fever immunization was first reported by Findlay & MacCallum [15] In 1938 these authors [17] reviewed their 4½ years experience with *various methods* of such immunization human immune serum plus neurotropic virus hyper immune horse serum plus neurotropic virus —in both these methods the virus component was made up in normal human serum—and thirdly an

attenuated pantropic strain of virus grown in tissue culture chicken embryo being substituted for mouse embryo in the culture and the amounts of immune serum being gradually reduced until only the chick grown virus suspended in normal human serum was employed Of 2 200 persons immunized by one or other of these methods 48 (2 2/) had attacks of acute hepatitis with symptoms resembling those of common infectious hepatic jaundice coming on at intervals of from two to seven months after immunization after 2 months 26 cases after 2 3 months 12 after 3-4 months 8 over 4 months and just under 7 months 2 Hepatitis occurred in those who had received immune serum plus virus and also in those who had received virus alone In a later review of their five years experience (1932 37) with these methods the same authors [16] stated that among 3 100 persons immunized 89 (2 9/) developed jaundice the aver age period between inoculation and onset of symptoms having been two to three months (minimum 36 days maximum just under seven months) Of the total vaccinated 1 000 received only attenuated virus made up with normal human serum to ensure its preservation the amount of such serum injected never exceeded 0 275 ml Between 1 August and 31 October 1937 all sera used were heated at 56°C for 30 minutes but among the 627 im munized during that period 13 cases (2 07/) of jaundice occurred In 1938 also Soper & Smith [61] reported that of 795 persons immunized in Brazil during 1936 37 with 17E plus hyperimmune monkey (rhesus) serum 20/ 30/ developed hepatitis from two to eight months after vaccination In connexion with the use of immune serum in sero virus methods of vaccination Findlay & MacCallum [17] had already drawn attention to the fact that cases of jaundice had followed the injection of human immune serum prepared in London and in Brazil (10 cases) of hyperimmune horse serum prepared in Paris (13 cases) and of hyperimmune monkey serum prepared in London (2 cases) the smallest amout of immune serum which had been followed by jaundice having been 10 ml Two extensive out breaks of hepatitis following the use of 17D vaccine have been reported One occurred in Brazil where according to Fox et al [20] among 19 000 per sons vaccinated with two lots of this vaccine prepared at Rio de Janeiro 886 developed hepatitis three to four months later and of these 22 died from liver atrophy The other by far the more serious occurred early in 1942 among United States Army troops who had been vaccinated with various lots of 17D vaccine prepared in New York in this outbreak according to Sawyer et al [54 55] there occurred some 28 000 cases with 62 deaths from acute or subacute yellow fever atrophy

As regards the etiology of hepatitis following yellow fever immuniza tion all available evidence in the opinion of Findlay & Martin [18] indicates that *in sero-virus methods the icterogenic agent—in* unusually thermo-stable filtrable virus—was introduced either in the pooled human immune

serum component or in the normal human serum invariably used (as stated by Findlay & MacCallum [17]) to make up the virus component of the prophylactic while in 17D vaccination the icterogenic agent was introduced via the *human blood serum used as a medium in the preparation of the vaccine up to 1942* In this latter connexion it is noteworthy that since 1942 when in the preparation of 17D vaccine the use of human serum as a menstruum for the chick-embryo virus was discontinued and sterile distilled water was employed in its stead there has been no case of hepatitis among the millions of persons immunized with 17D vaccine of the aqueous base (serum free) type

Category 2 Reactions Involving the Central Nervous System

The serious or delayed reactions now to be considered occur typically in from 12 to 15 days after immunization and present many characteristics of a meningo encephalitic syndrome They may or may not be preceded by moderately severe general reactions which appearing on the *fifth or sixth post vaccination day and lasting two to four days* have for symptoms some degree of fever (38° 38 5°C) headache mainly frontal of varying intensity backache pain in the limbs nausea anorexia and general weakness *According to Sorel* [56] *in his study of reactions following* the use of Laigret s attenuated neurotropic virus vaccine (three injection method) there ordinarily occurs on the sixth post vaccination day a first reaction—evidence of the viscerotropic activity of the virus strain employed —which clears up more or less rapidly Then on the 15th day when all appears to have returned to normal there is a recurrence of fever the temperature rising to 39°C accompanied by a particularly intense frontal headache Kernig s sign is present and there is markedly painful stiffness of the neck and vertebral column Lumbar puncture confirms these signs of a meningitic reaction the cerebrospinal fluid shows an increase in pressure and in albumen content and contains a hundred or several hundred cells per mm³ *These acute symptoms last five to six days then disappear* leaving behind only a feeling of weakness more or less marked which may if pronounced persist for a considerable time In short the symptoms at once most constant and nearly always *predominating are those* of a meningitic reaction more or less serious But in addition there are signs that the central nervous system has been attacked although frequently the meningitic tend to mask the encephalitic symptoms *In this connexion* Sorel recognizes besides the meningitic reactions (*formes meningees*) four types of reaction in which such signs of encephalitis as the following predominate

(*a*) transient motor paralyses convulsive seizures involuntary movements (*formes motrices*)

(*b*) hyperaesthesia (generalized or localized in certain regions) hypoaesthesia (along the area of distribution of certain nerves) sensation of cold in the extremities neuralgias (particularly facial and sciatic) (*formes sensitives*)

(*c*) mental confusion disorientation amnesia insomnia (*formes psychiques et mentales*) and

(*d*) lethargic manifestations irresistible desire to sleep (*formes lethargiques*)—a very rare type of which only two cases are recorded

A review of the relevant literature published during the twenty years period 1931 50 shows the reactions of the meningo encephalitic type to have been comparatively rare Their occurrence during that period and thereafter until 1954 is considered in the following paragraphs under the several methods of immunization listed earlier in the text and in that order

French neurotropic virus

(1) By inoculation together with immune serum

Sawyer[51] reported the occurrence among 88 persons treated with mouse adapted virus (at its 105th passage in mice) plus human immune serum of one case of probable encephalitis in a male aged 45 years 22 days after vaccination and one week after humoral immunity had been established The patient recovered but convalescence was protracted Darré & Mollaret[5] reported the occurrence in a female aged 25 years immunized with horse immune serum plus virus at its 148th passage in mouse brain of symptoms 11 days after inoculation acute meningitis epilepsy narcolepsy mental confusion intracranial hypertension The patient recovered after three or four weeks This case was classified by Sorel[66] as *forme psychique et mentale* No untoward reaction was shown by 102 other persons similarly immunized[68]

(2) By inoculation without immune serum

Single injection of fresh virus Laigret[39] reported that of 7 persons inoculated each with 1 10 000 suspension of fresh virus at its 134th mouse brain passage 3 developed untoward reactions 13 15 and 15 days respectively after inoculation and of these 2 had marked nervous symptoms jaundice haemorrhages and albuminuria

Laigret s three injection method (attenuated virus) Of 900 persons immunized by this method in Senegal two at Dakar experienced nervous

reactions According to Laigret[29] one of these reactions occurred in a male aged 32 years on the 17th day after his first injection and during the ten days of illness from which he recovered the patient presented the classical signs of a meningitic syndrome (Sorel s *forme meningee*) the second reaction was in a male aged 40 years who on the 16th day after his second injection developed not only a meningitic syndrome but also presented signs characteristic of Sorel s *forme motrice* From observations made in French West Africa Nicolle & Laigret[45] stated that of 10 000 persons immunized by three injections of dried virus two developed meningitis and one myelitis while in the experience of Mathis Laigret & Durieux[43] of 3 196 Europeans so immunized one suffered later from a meningitic syndrome and one from a myelitis with transitory paraplegia Laigret[31] then stated that among 12 000 persons immunized by this method there had been observed four cases with symptoms of meningitis two in Dakar one in Niger and one in Paris In recording the results of immunization by the three injection method practised at the Pasteur Hospital Paris Martin Rouesse & Bonnefoi[39] noted that of 38 persons who had received inoculations 24 were followed up and that among these 24 two showed serious reactions One occurred in a 6-year old child who on the 14th day after receiving his first and only injection (0 2 ml instead of the ordinary 0 5 ml) developed a meningitic syndrome which lasted one week until his recovery the second reaction was in a male aged 58 years who from the 14th day following his first injection showed symptoms of encephalitis of the type classified by Sorel as *forme psychique et mentale* convalescence was protracted

A case reported by Lhermitte & Fribourg Blanc[35] and by Barraux Montel & Bordes[3] is of special interest in that the individual immunized died from disseminated encephalomyelitis 14 months after having received his first and only injection This case was in a male aged 52 years whose inoculation had shortly been followed by fever headache and giddiness A few days later nervous symptoms appeared in the lower extremities—fatigue with loss of power cramps a feeling of cold and formication These symptoms cleared up after three weeks but three months later following an attack of dysentery the former condition in the legs reappeared walking became difficult there was a loss of sensation beginning in the legs and extending to thighs and forearms difficulty in passing urine and faeces was experienced From then until his death the patient showed progressive deterioration according to Sorel s classification the condition was one of the *formes motrices* pathological examination confirmed the diagnosis of encephalomyelitis

In his study of this Laigret method particularly in its application to French West Africa where between June 1934 and December 1935 the number of injections given had totalled 23 890 representing the prophy

lactic treatment of 9 592 persons of whom 5 699 had received the full course of three injections Sorel[66] noted inter alia that of the 721 post vaccination reactions for which the date of appearance was available 31 (4 30%) occurred between the first and fourth day after inoculation 637 (88 35%) between the fifth and seventh 20 (2 77%) between the 8th and 11th 24 (3 33%) between the 12th and 15th and 9 (1 25%) between the 16th and 21st As regards those untoward reactions delayed in their appearance until the 12th post vaccination day or later Sorel from accounts —published or communicated to him personally—giving all necessary clinical data classified the meningo encephalitic types of reaction in the manner previously indicated and in the 27 cases cited in his paper provided typical examples of reactions falling within the following categories *formes méningees* 6 cases *formes motrices* 7 *formes sensitives* 7 and *formes psychiques et mentales* 7 Of the 27 cases at least 18 occurred among persons vaccinated in French West Africa Sorel therefore felt himself unable to agree with the statement made by Laigret[31] that among 12 000 vaccinations against yellow fever there had been observed only four nervous reactions An explanation of this disparity was offered by Sorel—namely that many such reactions were probably never recorded because observations on inoculated persons were terminated by most workers on the seventh or eighth day after vaccination

Laigret s single dose method (attenuated virus) Vaccine of this type was administered by Mathis Durieux & Mathis[41] to 450 persons in French West Africa—400 Europeans and 50 Africans Among the latter there were no reactions of the 376 Europeans observed 226 showed no reaction but reactions occurred in 150 cases classified by the authors as mild (67) moderate (49) and prolonged (34) These prolonged reactions appeared in from 16 to 24 days after vaccination sometimes without a preliminary *febrile period and were characterized by elevation of temperature* (39 5° 40°C) intense headache pains in the joints and muscles photophobia hyperaesthesia itching stiffness of neck and sometimes jaundice The symptomatology was regarded as indicating involvement of the central nervous system but not to an alarming degree all cases recovered Details of another case showing involvement of the central nervous system were provided by Mathis Durieux & Mathis[4] in this case symptoms first appeared on the fourth post vaccination day (temperature 38 7°C head ache pains in limbs and back) lasted three days disappeared for a week then reappeared on the 14th day and persisted until the 26th day Stiffness of the neck insomnia hyperaesthesia main en griffe photophobia for six days and insupportable headache were the most pronounced features Jadin & Arnaldi[25] described reactions following the application of this method to 37 Europeans and to 2 220 Africans in the Belgian Congo In each of these groups there occurred three very serious delayed reactions

said by the authors to have been of the meningo encephalitic type and comparable with those described by Sorel[66] and by Martin Rouesse & Bonnefoi[39] In 1937 Dezest[6] gave a clinical account of the reaction he personally experienced after receiving his immunizing dose On the eighth post vaccination day appeared symptoms—headache backache stiffness of neck and vertebral column—which lasted 48 hours Thereafter for 16 days he suffered fugitive lumbar pains and then commencing on the 32nd day after inoculation and persisting for a month prior to convales cence there developed a condition in which fever (39 5° 41 5°C) intense headache anorexia signs of meningitis hyperaesthesia prostration delirium loss of consciousness bladder and rectal troubles and loss of concentration were present at one time or other

In 1937 Laigret[3-] reported that among 24 000 persons immunized in French West Africa only eight experienced really serious nervous reactions These reactions were mostly of the meningitic type but three developed myelitis one with transitory paraplegia Unfortunately Laigret in this paper did not indicate what proportion of the 24 000 was immunized by the three injection method and what by the single dose method nor what proportion of the eight reactions followed the application of each Clari fication on this point was however provided in 1953 by Laigret in a paper presented at the African seminar on yellow fever held at Kampala Uganda In this paper he stated that of a total of 25 000 persons vaccinated there were 12 cases of meningo encephalitis (0 50 per 1 000) and that of these 12 cases 5 occurred among the 9 000 persons vaccinated by the three injection method (0 55 per 1 000) and 7 among the 16 000 vaccinated by the single dose method (0 44 per 1 000) As regards the age incidence of these cases 3 occurred among 21 000 individuals over 15 years old at the time of their vaccination (0 14 per 1 000) and 9 among 4 000 children under the age of 15 years (2 25 per 1 000) incidence was therefore 16 times higher in the children than in the adults

(3) By scarification of the skin

Peltier[48] reviewing the experience with this method in French West Africa where by 1948 over 20 million vaccinations had been performed stated that two types of reaction had been observed an early one (see category 1 page 152) on the fifth or sixth post vaccination day in 10/ 15% of persons vaccinated and a delayed one of rare occurrence 12 to 15 days after vaccination consisting of a serious meningo encephalitic syndrome—indicating invasion of the nervous system by the vaccine virus—and clear ing up usually in from five to six days Recovery from these serious reac tions appeared to have been complete and without sequelae no fatalities were reported In connexion with the vaccinations just mentioned it is

of interest to note that in approximately 17 million of the total 20 million a mixture of yellow fever and smallpox vaccines was employed

A case of meningo encephalitis following application of Dakar vaccine by scratch has also been recorded by Kaplan & Gluck [27] The patient a 4½ year old child developed typical meningitic symptoms 11 days after vaccination with high fever headache somnolence a convulsive crisis and a state bordering on coma after two or three days there was sudden improvement leading to complete recovery

Again in 1947 Lartigaut & Moulies [34] published an account of further cases associated with the use of Dakar vaccine Of four children belonging to the same family who had been vaccinated on 5 December 1946 three experienced reactions The first an 8 months old baby girl developed a feverish condition on the 12th post vaccination day The second a 2 year old boy exhibited also on the 12th post vaccination day signs and symptoms of a very severe attack of meningitis with involvement of the ocular nerves The third an older boy suffered from headache on the fifth and sixth post vaccination days and thereafter remained normal until the 17th post vaccination day when there appeared signs of meningitis accompanied by the vomiting of blackish material and by convulsions of a fleeting nature Recovery was complete in all three cases

Durieux (see page 46) refers to the reactions which have been observed *to follow the use of Dakar vaccine in French West Africa* and in French Equatorial Africa In the former territory where during the past 12 years some 45 million vaccinations or revaccinations have been carried out reactions have been of two types one a general reaction appearing regularly on the fourth or fifth day after vaccination (see under category 1 page 153) the other a delayed reaction with onset on or after the tenth post vaccination day Of rare occurrence such delayed reactions took the form of a meningo-encephalitis mostly of moderate intensity characterized by fever headache stiffness of the neck Kernig s sign present convulsions in children irritability hypertension in the cerebrospinal fluid with the albumen content and the number of cellular elements in that fluid higher than normal Accidents of this type occurred singly and never constituted foci in all they numbered but a few score they usually ended in recovery without sequalae fatal cases were exceptional Noteworthy however with respect to this territory is the fact that early in 1950 about twenty cases of meningo encephalitis occurred among children in the Dakar area and among these there were two deaths These cases were scattered through several districts in the town were spread over a period of several months *and appeared a short time after group vaccination against yellow fever* had been performed Half the cases however had not been vaccinated and all attempts to isolate the vaccine virus by inoculation of laboratory animals with nervous tissue taken from fatal cases proved unsuccessful

It was concluded therefore that the yellow fever vaccination had activated some encephalomyelitogenic virus latent in the affected children In French West Africa in general it had been sometimes possible to ascribe the occurrence of such untoward reactions to the existence of concomitant affections such as malaria trypanosomiasis or relapsing fever in other cases the etiology could not be ascertained but although the research undertaken to detect the virus had had a negative result it appeared reasonable to accept the immunizing virus as the causative factor (see under part III page 182)

From the foregoing it emerges that the nervous reactions observed in French West Africa have invariably appeared as isolated cases and have never constituted foci In French Equatorial Africa however during September 1944 there occurred in the Brazzaville area where 102 000 vaccinations had been carried out with combined yellow fever and smallpox vaccines 102 cases of meningo-encephalitis with 18 deaths—the over all case rate having been therefore 1 per 1 000 and the mortality rate 0 15 per 1 000 These reactions with onset from 10 to 20 days after vaccination occurred over a period of three weeks and appeared in the form of foci confined to a particular area Of those affected 62 (60%) were under 6 years of age Appropriate investigations failed to yield any results on which conclusions as to the precise etiology of these nervous complications could be based Inoculation of test animals did not reveal the presence of the yellow fever vaccine virus and inasmuch as a large number of the vaccine batches used in Brazzaville had been utilized for vaccination in other territories without untoward effects it was agreed that certain vaccinated individuals had been harbouring latent viruses the pathogenicity of which had been reinforced by the yellow fever vaccination (but see under part III page 182)

In 1951 and in early 1952 two serious outbreaks of encephalitis were also reported to have followed the use of Dakar vaccine in Costa Rica and in Nigeria resp ctively

In Costa Rica this vaccine was employed in 1951 to assist in coping with the wave of jungle yellow fever which in June of that year had crossed from Panama and was proceeding in a north westerly direction Contrary to expectation and previous experience a large number of reactions of all grades of severity resulted from the use of two of the vaccine lots employed and among these reactions there were 12 cases of encephalitis in children with three fatalities From an unpublished preliminary report by Eklund in 1952 it transpires that all 12 cases developed within a nine day period and in from 8 to 22 days after vaccination Symptomatology was compatible with an acute encephalitis severe headache high fever vomiting convulsions drowsiness stupor or loss of consciousness the spinal fluid examined in five of the cases showed an increase in the cell

count As indicated in table II the ages of the cases were uniformly dis tributed between the two extremes of 13 years and of nine months and among them there was an unusual preponderance of females

TABLE II CASES OF POST VACCINATION ENCEPHALITIS IN COSTA RICA 31 AUGUST — 9 SEPTEMBER 1951

Case o	Age (yea s)	Se	Day of o s t of a te symptoms afte ac i natio	Res lt
1	6	m l	8-9	reco e d
2	3	fem le	13	di d
3	10	fem le	14	d ed
4	5	fem le	22	eco e ed
5	9 12	fem le	12	eco e ed
6	13	fem le	9-12	cove d
7	2	female	12 15	died
8	8	fem le	10-13	cove d
9	7	m l	10 13	eco e d
10	10	m l	12 15	reco e ed
11	15/12	fem le	12 15	reco ered
12	3	fem le	15	re o e ed

It is noteworthy that 11 of the above cases followed the use of one par ticular lot of vaccine of that number six were inhabitants of an area (La Fortuna) which had not been reached by the yellow fever wave The twelfth case on the list was immunized (at Los Chiles near the Nicaraguan border) with a different lot of Dakar vaccine in this instance the child showed on recovery from the encephalitis a completely changed per sonality

In addition to the 12 cases in Costa Rica there occurred a case of probable encephalitis in Honduras where 1 200 persons had been immu nized with yet another different lot of Dakar vaccine In this instance a 3 year old boy had slight fever on the seventh and eighth days after vac cination on the ninth day the temperature rose to 40°C there were two convulsions and the patient became stuporous for the next five days he remained stuporous with a temperature of about 39 5 C In a further report Eklund [11] mentioned that within the same nine day period a thir teenth case had occurred in Costa Rica and that there had been a second —*this time a well defined case*—in Honduras

In Nigeria according to the *Annual General Report for 1951 52* of the West African Virus Research Institute Lagos [78] there occurred towards the end of 1951 an outbreak of yellow fever in the Udi Division Onitsha

Province—part of the Eastern Province in which the town of Enugu is situated The affected area some 20 miles (30 km) square is centred about 20 miles west of Enugu town Protection of the threatened population was effected with Dakar vaccine Among the first 20 000 persons so immunized two cases of encephalitis were stated to have occurred in boys aged 5 and 7 years 12 and 14 days respectively after vaccination Among a further 80 000 vaccinations then performed no case of encephalitis was reported Thereafter between 14 January and 7 February 1952 mass *vaccination was carried out in Enugu where of 42 400 persons immunized* 19 358 were under the age of 10 years Between 28 January and 21 February that is within a 24 day period 83 cases of encephalitis were admitted to Enugu General Hospital and of that number 32 died—73 cases with 29 deaths among children under 10 years of age 10 cases with 3 deaths in persons over that age

The age incidence of cases and deaths is shown in the following tabulation

Age (years)	*Cases*	*Deaths*	*Case fatality (%)*
0 1	33	13	39
2-4	25	12	48
5 9	15	4	26 6
10 and over	10	3	30
Total	83	32	38 5

The over all case rate in vaccinated children under 10 years was 3 77 per 1 000 and the death rate in this group was 1 498 per 1 000 in those over 10 years of age these rates were respectively 0 434 and 0 13

The time interval between vaccination and onset of symptoms in respect of 54 out of the 83 cases of encephalitis may be summarized as follows

Interval in days	7	8	9	10	11	12	13	14	15	16	17	19	23
Number of cases	1	1	3	4	9	13	7	6	1	5	1	2	1
Median	11 7 days												

The symptomatology is described thus Onset sudden with fever headache and convulsions Convulsions continued and signs of encephalitis developed rapidly Patients became irritable stuporous delirious and incoherent Spasms of muscles and choreiform movements were seen head retraction was not a conspicuous feature Temperature was high and usually sustained Respiration rate was invariably increased without however abnormal signs in the chest In some cases the tongue was typical

of that described in yellow fever About one week from onset the temperature fell convulsions ceased and the patient gradually recovered When death occurred it was usually within the first week sometimes within the first two or three days

In regard to these cases of encephalitis most occurred in areas which had not been touched or had been affected but little by the outbreak of yellow fever prevailing at the time and cases followed the use of three different lots of Dakar vaccine although one lot was particularly associated with their appearance

In connexion with the nervous reactions which followed the systematic application of Dakar vaccine in the Brazzaville area of French Equatorial Africa in Costa Rica and in the Enugu Town area of Nigeria it is of interest to observe that in each of these territories they commonly appeared in the form of foci confined to a particular area Moreover all 102 encephalitis cases in the Brazzaville area developed within a 21 day period and in from 10 to 20 days after vaccination all 13 cases in Costa Rica developed within a 9 day period and in from 8 to 22 days after vaccination all 83 cases in Enugu Town developed within a 24 day period and with respect to the 54 cases for which the precise date of onset could be given in from 7 to 23 days after vaccination In all three territories the number and severity of these reactions led to a discontinuance of the employment of Dakar vaccine as a measure of prophylaxis in French Equatorial Africa however routine immunization with that vaccine was resumed in 1945 since when no untoward reactions have been observed to follow its use

Derivatives of the Asibi strain 17E

By inoculation together with immune serum

No reactions involving the central nervous system have been reported to follow the application of this method

Derivatives of the Asibi strain 17D

(1) By inoculation without immune serum

Theoretically the use of a strain which on laboratory demonstration has been rendered essentially avirulent neurotropically as well as viscerotropically while still retaining in large measure its antigenic potency should preclude the occurrence of cases of severe reactions involving the nervous system The validity of this assumption appeared to have been confirmed by the results of the first large scale field trial undertaken in

Brazil with this virus as vaccine during 1937 when 59 532 carefully observed vaccinations were carried out and reported on by Smith Penna & Paoliello [59] As these results failed to show any contra indication to its use mass vaccination with 17D was thereafter performed particularly in Brazil where by 1938 according to Soper & Smith [65] over half a million persons had been so immunized and where by 1940 over two million had been protected by this means Between 1937 and 1940 no nervous reaction following immunization with 17D vaccine had been recorded in the literature other than one referred to by Soper & Smith [65] as having occurred one month after vaccination, with *definite meningeal signs the relation* of which to vaccination was however considered to be very doubtful

In 1941 however there appeared in various localities in Brazil cases of undoubted encephalitis after immunization with certain lots of vaccine prepared from several substrains of the original 17D virus The results of an investigation made into this unexpected occurrence were reported by Fox et al [19] These authors classified as encephalitis those cases which presented the following signs or symptoms onset market by violent headache often not localized and not relieved by any medication or by spinal puncture temperature rising rapidly to 40°C or over persisting without *remission and subsiding gradually* anorexia usually complete and accompanied by nausea and vomiting Of the manifestations more particularly referable to the central nervous system the most common suggested meningeal irritation nuchal pain and rigidity positive Kernig's sign Other manifestations included marked drowsiness or somnolence often leading in children to deep torpor transient convulsive seizures individual muscle spasms and tremors—particularly in children other disturbances in motor function transient localized weakness or spasticity of an extremity muscular incoordination laryngeal paralysis delirium calm or excited alternating with periods of somnolence insomnia

In the area most extensively studied where in the immunization of 55 073 persons there had been employed seven lots of vaccine deriving from one or other of two sister seed lots and containing therefore practically identical virus components 199 cases (0 36/) of encephalitis occurred after an average incubation period of 12 7 days The average total duration of illness was 12 days nine of which were spent in bed in a greatly weakened condition the patients began their convalescence which was usually protracted In only two cases however did serious disabilities persist two children 3 and 7 years of age when last seen on the 49th and 71st days of illness still exhibited unilateral brachial paralysis an uncoordinated gait and evidence of mental retardation There was one fatality—in a child of 2 years who died on the fourth day of illness following a period of alternating coma and convulsive seizures

Recognition of the probable relationship of the encephalitis to the entire series of vaccine lots used in the area surveyed—for all seven lots seemed to have been equally concerned in the production of the 199 cases—made apparent the need for choosing a new 17D substrain one which would be free from encephalitogenic properties realization of this desideratum could of course be demonstrated only by the results obtained from an adequately controlled field trial Four new substrains were therefore selected and corresponding lots of vaccine prepared for use in an area different from the first For purposes of control one vaccine lot which had previously been used in the first area and another lot prepared from uninfected chick embryos were included in the trial These six lots of vaccine were then employed simultaneously over a three week period to inoculate some 19 000 persons

Among the 14 770 persons examined for post vaccination reactions there were 55 (0 38%) cases of encephalitis and of these 49 (1 34%) occurred among the 3 644 persons inoculated with the vaccine lot which had been already used in the first area of these 49 cases 36 were in children between one and 14 years of age

As regards the time of appearance after vaccination cases of ence phalitis did not occur at all before the sixth day did not reach their peak of incidence until the 9th to the 11th day and continued to occur with significant frequency up to the 20th day the average incubation period was 12 3 days In detail the times of appearance of these 55 cases are 6-8 days after vaccination 6 cases 9 11 days 23 cases 12 14 days 14 cases 15 17 days 6 cases 18 20 days 5 cases and 21 23 days 1 case

As regards age it will be seen from the following figures that cases of encephalitis were more frequent in the younger age groups 1-4 years 3 cases 5 9 years 20 cases 10 14 years 17 cases 15 19 years 6 cases 20-29 years 5 cases 30-39 years 1 case and 40 years and over 3 cases

In connexion with the foregoing thorough investigation by Fox and his collaborators [19] indicated that the vaccine virus itself was responsible for the outbreak and not any extrinsic agent The sudden alteration in character of the 17D virus seemed to have occurred during a very small number of subcultures away from the parent strain The technique of vaccine production was therefore altered in 1942 so that all the vaccines to be used for immunization were initiated from primary and secondary seed lots of known character only

The standardization of 17D vaccine preparation consequent on the adoption of this seed lot system seemed to have achieved the desired result for during the next 10 years no case of encephalitis following the use of 17D vaccine alone as a prophylactic was recorded in the literature

In a personal communıcatıon dated 22 June 1953 however Dr P Lepıne reportıng on the results of 17D vaccınatıon at the Instıtut Pasteur Parıs stated that durıng 1952 53 there had occurred among 1 800 chıldren less than one year of age at the tıme of their vaccınatıon five cases of menıngo encephalıtıs (0 278%) Of these five cases four occurred among 1 000 ınfants under 6 months of age (0 40%) while one occurred among 800 ınfants between 7 months and 12 months old (0 125%) All five cases recovered No case of menıngo encephalıtıs was observed among 40 000 adults ımmunızed durıng the same perıod with the same vaccıne used to vaccınate the 1 800 ınfants

The maın partıculars of the five cases are summarızed ın table III

TABLE III SUMMARY OF OBSERVATIONS ON 5 MENINGO ENCEPHALITIC REACTIONS FOLLOWING VACCINATION WITH 17D VACCINE AT THE INSTITUT PASTEUR PARIS IN 1952 53

Case no	Age (mo ths)	Sex	Incu bat on period (days)	Clinical p cture	Duration	*Result of sero-protect on test*
1	7	female	19	convulsi e seiz ures the hemi pl gia CSF 12 cells per mm		++ = *250 LD₅₀*
2	1/	male	11	repeated convul sive seiz res (6 per day) left sided	48 hours	*not test d*
3	1	male	12	repeated con ul *sive seizures*	48 hou s	negat ve = *less* than 25 LD
4	6	male	12	certain s gns of *encephal t s* t mpe atu e 38 *39 C om t g* CSF 120 cells *per mm*	5 days	not tested
5	*4*	*female*	*10*	*hosp talized* 5 days for h gh tempe*ratu e* (40°C) lumbar punctu e on 13th day (sl ght p essu e ov r lo ta elle) CSF 150 cells pe mm ret rn to normal on 16th day	5 days	1st test (16th day after acc nat on) = 25 LD₅₀ 2nd test (27th day after acc nation) = 200 LD₅₀

In these cases the perıod between vaccınatıon and the onset of symptoms varıed from 10 to 19 days the duratıon of ıllness from 48 hours to 5 days One a 7 months old Afrıcan chıld with onset of symptoms on the 19th post vaccınatıon day had by then developed a satısfactory *ım*munıty response to the vaccınatıon

Again the occurrence of a sixth case at the end of 1953 was reported from France by Lartigaut & Couteau [33] This case followed the vaccination of a 4 months old baby boy on 10 December The baby remained in perfect health until the eighth post vaccination day when he showed a disinclination for food developed a high temperature (39 4 39 8 C) had an attack of vomiting and continued drowsy throughout the day On the ninth post vaccination day his temperature was still high and in the morning of that day he had a shivering fit after his breast feed in the early afternoon he had a generalized convulsion lasting three to four minutes thereafter he remained rigid for nearly one hour at the end of that time he was very pale and exhausted and his respirations were irregular later in the afternoon he was admitted to hospital Two hours after admission his temperature was 38 7°C he was of good colour and desirous of food his respirations and reactions were normal there was no stiffness of the neck Kernig s sign was absent physical examination revealed no abnormality in chest abdomen or fontanelle on lumbar puncture the cerebrospinal fluid was found to contain 0 80/ albumen and 238 cells per mm³ (lymphocytes 69/ polymorphonuclear cells 23 monocytes 8/) On the tenth post vaccination day the temperature fell to normal and from that time the child s recovery was uneventful

Three further cases following 17D vaccination were reported in 1953 54 —this time in England The first case followed the vaccination on 10 November 1953 of a baby girl aged 5 weeks On the 11th post vaccination day the child who for two days previously had been disinclined for food exhibited marked twitching on the right side of the body and a rolling upwards of the eyes The twitchings occurring throughout that day at 5 to 10 minute intervals gradually became more violent and of longer duration and occasioned the child s admission to hospital on the 13th post vaccination day On admission the child had a temperature of 101 8 F (38 8°C) a pulse rate of 152 and a respiration rate of 66 Several fits at about 10 minute intervals were observed—fits which ordinarily started on the right side involving the face mouth and eyebrows and the right arm and leg but occasionally were more generalized involving both arms and both legs Physical examination revealed nothing abnormal in heart lungs abdomen throat eyes or ears knee jerks present and equal no papilloedema in fundi full movement in limbs Blood examination showed a white cell count of 31 000 per mm³ (polymorphonuclear cells 59 / lymphocytes 29/) The cerebrospinal fluid was clear colourless and not *under pressure* *contained protein 90 mg / sugar 60 mg* *and chlorides* 690 mg / showed a faint increase in globulin a white-cell count of 117 per mm³ (polymorphonuclear cells 40/ lymphocytes 60 /) no acid fast bacilli no bacteria in gram film and no growth on culture X ray examination of the skull showed no evidence of any bone injury The

progress of this case to complete recovery was as follows the temperature fell to normal two days after the child s admission to hospital the twitch ing after being controlled by intramuscular paraldehyde and intramuscular sodium amytal soon decreased and was only present when the child was moved by the end of her stay in hospital (19 days after admission) the child was not twitching at all although on occasion athetoid movements were observed One month after discharge from hospital the child was found to be perfectly well without any sequelae This case which occurred at Bath was reported by Smith [63]

The second case occurred early in 1954—*this time following the vac cination of a 13 weeks old baby boy* On the ninth and tenth post vaccina tion days the child was irritable and disinclined for food he was feverish for 18 hours and drowsy for six hours before admission to hospital on the 11th post vaccination day *On admission he was found to have a tem* perature of 102 6°F (39 2°C) a bulging fontanelle rigidity of the neck and a positive Kernig s sign there were no other abnormal physical signs Blood examination showed a white cell count of 18 800 per mm³ (lymphocytes 60/ polymorphonuclear cells 34% *monocytes 6%) there was no growth* on blood culture The cerebrospinal fluid showed a pressure of 205 mm Hg contained protein 120 mg % and sugar 60 mg % had a cell count of 460 per mm³ (lymphocytes 50% polymorphonuclear cells 50/) and revealed no organisms in film and no growth on culture Treatment was 10 000 units of penicillin intrathecally on admission systemic penicillin streptomycin and Gantrisin for 10 days The infant made an uneventful recovery the signs of meningeal irritation disappeared after 24 hours by the fourth day in hospital he was apyrexial and by the sixth day without abnormal physical signs This case which occurred in London was reported by Haas

The third case followed the vaccination of an 8 weeks old baby boy *The child who had been inoculated in Nigeria on 11 September 1954* and had returned to England one week later was admitted to hospital on 30 September He had been listless for five days prior to admission had a slight convulsion the evening before admission and two serious convul *sions the day of admission On clinical examination* the child was drowsy his rectal temperature was 101°F (38 3°C) the anterior fontanelle was normal On lumbar puncture the cerebrospinal fluid was found to con tain 133 leucocytes per mm³ (mostly lymphocytes) protein 140 mg % *sugar 55 58 mg % and chlorides 700 mg /* no growth was obtained on culture Blood examination showed a white-cell count of 13 500 per mm³ (lymphocytes 54% polymorphonuclear cells 42/ hyaline bodies 4/) X ray examination of the chest revealed no abnormality The main treat *ment was 50 mg of Aureomycin orally every 6 hours* and 0 13 g of chloral hydrate every 6 hours The temperature fell to normal on 3 October and

remained so until the child s discharge from hospital on 12 October when recovery was complete This case which occurred at Bexhill on Sea Sussex was reported by Scott [68]

Both in France and in England the vaccines associated with the occur rence of the nine cas s just described were prepared from lots of seed virus procured in 1946 from the Yellow Fever Laboratory of the Rocke feller Foundation New York —lots which in accordance with th provi sions of Article XI (10) of the International Sanitary Convention for Aerial Navigation 1944 had satisfactorily passed the monkey safety and im munity tests prescribed by the United Nations Relief and Rehabilitation Administration in *Standards for the Manufacture and Control of Yellow Fever Vaccine* (see page 205) Moreover both at the Institut Pasteur Paris and at the Wellcome Research Laboratories Beckenham Kent the vaccines concerned were prepared solely from the first passage in eggs from these tested seed lots and not as was the case with the vaccine which occasioned the 1941 outbreak of enc phalitis in Brazil from virus which *had gone through serial passages in eggs*

It has been mentioned previously that in the ten year period following 1941 no case of encephalitis associated with the use of 17D vaccine when administered alone had been recorded in the literature According to Dick & Horgan [8] there had on the other hand been two cases reported in the United Kingdom during that period to follow vaccination with a combined prophyla tic—vaccination with yellow fever and smallpox vac cines The first of these cases was in an adult male who inoculated with yellow fever vaccine 13 days after primary vaccination developed ence phalitis the following day and died five days later The second case was in a child aged 6 years who was given a yellow fever inoculation three days after primary vaccination definite encephalitis developed on the eighth day after va cination but the child recovered In the words of the authors

It cannot be stated whether the above reactions might not have occurred if no yellow fever vaccine had been given Seven cases of post vaccinial encephalitis were reported during the same year

(2) By scarification of the skin

Following the application of this method to 3 961 Africans no unto- ward reactions were observed by Hahn [23] or by Dick [7]

Note It will be evident from the foregoing that in proportion to the total number of persons vaccinated against yellow fever the incidence of serious nervous reactions has been extremely low The fact that they have occurred at all howe er serves as a reminder that no method of act ve immun zat on not even one so su cessful as yellow fever vaccination has proved to be ne e er entirely free from risk

III ETIOLOGY OF REACTIONS INVOLVING THE CENTRAL NERVOUS SYSTEM

Views on Etiology of Encephalitis Following Use of Different Viruses

French neurotropic virus

Cases of encephalitis in man have followed the use of this virus both when administered together with specific immune serum and when administered alone and explanation of their occurrence has primarily been sought in an etiological factor present in the vaccine material itself Thus inasmuch as virus vaccines of this type derive from infected mouse brain the possibility of a filtrable virus accidentally present in the mouse brain being introduced into the immunizing vaccine and proving pathogenic to man was suggested by Darre & Mollaret [5] as the causal agent Sawyer [51] reviewing theories of causation also mentioned this possibility Sorel [66] considered the encephalitis to be of uncertain origin it might be due to a spontaneous mouse virus or to the immunizing virus itself On the other hand Barraux Montel & Bordes [3] suggested that instead of a mouse virus or the yellow fever virus one of the ultra viruses capable of producing encephalomyelitis in man—e g Japanese B encephalitis virus—might well play the causal role such a virus pre existing in the human subject might be stimulated into activity by the yellow fever vaccination In 1937 Laigret [32] assessing the position at that time referred to the suggestion that the condition might be attributed to the Traub Armstrong virus which sometimes infected the breeding-cages of mice and might therefore contaminate the vaccine virus He considered that the hypothesis was of interest but that research had not yet led to any conclusive findings in its support It had to be observed that the Traub-Armstrong virus—probable causal agent of certain lymphocytic meningitides of man—showed itself in mice by signs particularly paralysis which would be easy to detect *No infection of this nature had occurred in his laboratories* Moreover meningo encephalitic reactions did not appear in series among persons vaccinated with the same mouse brain He believed then that the most likely explanation of post vaccination encephalitis lay in an exceptional attack on the central nervous system by the yellow fever virus adapted to mouse brain Martin Rouesse & Bonnefoi [38] also considered their cases to have been due to the immunizing virus rather than to a spontaneous mouse virus Peltier [48] summarizing the results of over *20 million* vaccinations in French West Africa by the Dakar method ascribed the small number of delayed reactions which occurred between the 12th and 15th post vaccination days to an invasion of the nervous system by the immunizing virus Mathis [40] in defending the use of the French neurotropic virus

in immunization suggested that the few cases of encephalitis which followed the administration of the mouse brain yellow fever virus vaccine were due to an actual infection with the vaccinating strain and not to a demyelinization from the injection of heterologous brain tissue

These views apportioning responsibility to the immunizing virus itself harmonize with those of other workers who have on laboratory evidence at various times expressed the belief that the increased neurotropism of the mouse adapted French strain renders it potentially dangerous for human vaccination Thus Findlay [12] observing the correlation of the development of immunity in monkeys with the circulation of living neurotropic virus in the peripheral blood stream emphasized this danger because of the possibility that the barrier between the blood stream and the brain may be broken down and the central nervous system invaded by the virus In 1935 Theiler & Whitman [8] found that when administered extraneurally to rhesus monkeys small doses of neurotropic virus with inadequate immune serum were more likely to give rise to encephalitis than was a large dose and that encephalitis did not develop if the amount of immune serum was sufficient to prevent the virus from entering the circulation All three monkeys injected subcutaneously with a 1 10 000 000 dilution of neurotropic virus (the minimum infective dose for monkeys and representing 2 MLD for mice) and which showed circulating virus even in the presence of small quantities of immune serum—0 02 ml 0 004 ml and 0 008 ml per kg body weight respectively—died of encephalitis while all three monkeys injected with the same quantities of serum but with 1 100 dilution of virus survived and developed active immunity Monkeys injected with 0 1 ml of immune serum per kg of body weight together with either 1 10 000 000 or 1 100 dilution of virus survived and became solidly immune These authors [77] reported also in 1935 that fatal encephalitis in rhesus monkeys inoculated extraneurally with neurotropic virus occurred particularly among younger animals and that such occurrence inclined them to the view that the blood brain barrier of young animals is more permeable to certain agents than that of adults In this respect they pointed out that whereas young mice repeatedly develop encephalitis after the subcutaneous inoculation of French neurotropic virus the incidence of encephalitis following this route of administration decreases with age and encephalitis is rare among adult mice after extraneural inoculation of the virus In 1937 Theiler & Smith [5] stated that in rhesus monkeys encephalitis followed the administration of very large as well as of very small doses of French neurotropic virus but was more apt to follow infection with extremely small doses In their experiments one monkey inoculated subcutaneously with 0 01 g of mouse brain virus died of encephalitis on the 11th day while three rhesus inoculated by the same route each with 0 000 001 g died of encephalitis on the 10th 11th and 24th day

In Nigeria where following the immunization of 42 000 Africans with Dakar vaccine 83 cases of encephalitis had occurred within a 24 day period—with a time lapse of 7 to 23 (mostly 10 to 13) days after vaccination—the findings of investigations made to discover the cause of the outbreak were published in the annual report for 1951 52 of the West African Virus Research Institute [8] and elsewhere in greater detail by Macnamara [38] These findings included the results obtained from the laboratory examination of the brains of five cases of encephalitis brought for autopsy within one hour of death

Sections of brain tissue were triturated and inoculated intracerebrally *into mice* mice *inoculated* with brain tissue from four of the five cases became sick between the sixth and eighth day after inoculation showing rapidly developing paralysis and ensuing death Further passages of brain material from sick mice were made and specificity tests performed on the four strains of virus isolated these tests showed that the virus was yellow fever virus that it was a neurotropic strain and that it was uncontaminated by any other virus pathogenic for mice by the intracerebral route To quote Macnamara [38] The virus recovered from mice becoming sick was shown to be that of the yellow fever vaccine by the inoculation of rhesus monkeys and by specificity tests Yellow fever vaccine virus was therefore shown to have been isolated from the brain sections of four persons who had died of encephalitis 14 14 18 and 19 days respectively after their immunization by the Dakar method These deaths occurred in children aged respectively 11 months 2½ months 7 months and 7 years In further connexion with this inquiry into the cause of the encephalitis cases in Nigeria Macnamara [38] said

> Epidemiological and virological evidence does not point to the presence of a concomitant or contaminating viral infection and the histological picture excludes perivascular myelinoclasis Moreover the minute amount of mouse brain scratched into the skin could hardly be considered to be the cause of an allergic demyelinization and encephalitis following yellow fever vaccination when a chick-embryo preparation of 17D was employed [19] still further indicates that the encephalitis is a result of the direct action of the yellow fever virus on the host

17D virus

Between 1937 and 1951 inclusive the only references in literature to reactions with involvement of the nervous system following immunization with 17D vaccine were one made by Soper & Smith [65] in *1938* on a reaction with definite meningeal signs the relation of which to vaccination was considered very doubtful and one made by Fox et al [19] in their 1942 report on the occurrence of encephalitis in a small proportion (0 36/ 0 38/) of persons immunized with one or other of certain lots of 17D vaccine *in* Brazil during 1941

In so far as the 1941 occurrence is concerned a thorough investigation by Fox and his collaborators indicated that the immunizing virus itself was responsible and not any extrinsic agent The yellow fever virus was alone demonstrated in the inocula moreover the sera of convalescent cases were shown to contain neutralizing antibodies against yellow fever virus but none against the viruses of St Louis encephalitis Japanese B encephalitis Eastern and Western equine encephalomyelitis lymphocytic choriomeningitis and West Nile disease During this investigation it was found with respect to one substrain used for the vaccination of 3 644 persons among whom 49 (1 34%) cases of encephalitis had later developed that a small number of passages away from the 17D parent strain had brought about an undesirable alteration in the pathogenicity of that particular substrain—namely an increase in neurotropism evidenced by its producing in rhesus monkeys when inoculated intracerebrally an unusually high incidence of encephalitis Frank encephalitis in three instances fatal was observed in 7 (12%) of 60 animals in the series and when all possible cases including monkeys with voice changes and transient weakness were considered the number was raised to 17 monkeys or 28% of the series For comparative purposes it should be stated that after the intracerebral inoculation of 245 monkeys with the 17D substrain used prior to 1940 for vaccine preparation in Brazil only from 4 1% to 5 7% developed encephalitis Further studies by Fox & Penna [21] confirmed the finding that at certain passage levels variation in the pathogenic properties of the 17D virus may take place as a result of altered cultural environment Such variation in pathogenicity of different substrains of the 17D virus and the consequent variation of the response in man inoculated with these substrains led to the standardization of the manufacture of 17D virus vaccine This standardization entailed the preparation of a large seed lot of virus which after having been thoroughly tested in monkeys was stored in the refrigerator from this seed lot successive batches of vaccine were made Since the establishment of the seed lot system in the production of 17D vaccine there had been no further occurrence of encephalitis following 17D vaccination until 1952 54 when between August 1952 and December 1953 six cases were observed in France and between November 1953 and October 1954 three in England—all nine in children under one year of age of whom eight were less than 6 months old

Views and comments on the etiology of these unexpected cases follow Smith [60] suggests two possible explanations of their occurrence either a hypersensitivity to the prophylactic in small infants—a hypersensitivity not shared by adults—or an increased neurotropic affinity of the virus in spite of limited serial passage Neurotropic affinity of the yellow fever virus appears to Haas [32] to have been a very likely cause of the aseptic meningo encephalitis in the case he described In certain individuals par

ticularly in young children hypersensitivity to 17D vaccine is considered by Lartigaut & Couteau[33] to be responsible for such nervous reactions these authors stress the need for keeping close watch on the 17D substrains used for vaccine preparation in order to ensure the freedom of such immunizing substrains from any undesirable increase in their neurotropism

As regards the suggestion that an increase in neurotropism may have been the responsible factor however it can be stated that all nine cases followed inoculation with vaccine prepared from first passage in eggs from lots of seed virus which had satisfactorily passed the prescribed monkey safety test their etiology in contrast with that of cases in the 1941 outbreak of encephalitis in Brazil could hardly be ascribed therefore to an increase in the neurotropism of the vaccine virus

In further connexion with the possible etiology of the nine cases under consideration it has been contended that a very low dosage of the vaccine virus may result in tardy immunization and that meningeal or cerebral involvement may occur before antibodies have developed it has been ascertained however that the dosage employed in the inoculation of those cases for which precise data are available contained an amount of active virus in excess of 500 MLD—the minimum requirement for the satisfactory immunization of man

The hypothesis has also been advanced that in infants less than one year old—and all nine cases belong to that age group—the antibody response to yellow fever vaccination may be negligible and in certain instances inadequate therefore to prevent the vaccine virus from entering the central nervous system and by its multiplication there giving rise to encephalitis In this regard however it has been stated by Stuart in a paper presented at the African seminar on yellow fever held in 1953 at Kampala Uganda that while it is quite possible that the response of a very young infant to a yellow fever vaccination may be negligible such data as are to be found in the relevant literature are insufficient either to confirm or to refute the hypothesis

Again in view of the possible bearing primary smallpox vaccination might have had on the etiology of these cases inquiries were made as to whether in fact such vaccination had been carried out at about the same time as the yellow fever vaccination and if so as to what the time intervals had been between the administration of these two live virus vaccines Results of these inquiries showed that with respect to the three cases occurring in England primary smallpox vaccination (a) had not been carried out in the case reported by Scott[58] (b) had indeed been performed in the case reported by Smith[60] but not until 25 days after the baby girl had left hospital cured of her meningo encephalitis and (c) had in the case reported by Haas[2] been carried out one week after the yellow fever

vaccination and two days before the onset of nervous symptoms With respect to the six cases occurring in France comparable data were not available at the time when the inquiries were made it is learned however that had smallpox vaccination been performed it would *not* have been within 21 days of the yellow fever vaccination

Finally inasmuch as meningo encephalitis has been known to occur in infants who have not been subjected to any immunizing procedure the possibility that the encephalitis in the nine cases now described was coincidental and not the result of yellow fever vaccination cannot be overlooked

Views on Etiology of Encephalitis Following Yellow Fever Vaccination in General

On the etiology of encephalitis in general after yellow fever vaccination the following views were expressed at the WHO sponsored African seminar on yellow fever held at Kampala Uganda during September 1953

Laigret regarded the febrile reaction on the 6th post vaccination day and the nervous reaction on the 11th to 15th day as two phenomena independent of each other The febrile reaction was certainly due to the vaccine virus for such a reaction had never been observed in the case of persons revaccinated The nervous reaction might on the other hand be caused by the virus constituting the vaccine by a virus contaminating the vaccine or by a virus pre existent in the body of the vaccinated person With regard to the last mentioned possibility it was known for example that the virus of lymphocytic choriomeningitis could be already present since spontaneous cases of choriomeningitis in man and particularly in children had been reported This virus as well as other viruses with encephalitogenic properties could undoubtedly exist in man as asymptomatic infections and might by association with the yellow fever vaccine virus cause accidents of vaccination Differences in the nature of these other viruses could explain the varying degrees of severity of post vaccination encephalitis experienced in countries in which such viruses were prevalent or endemic His belief that viruses in association constituted the principal etiological factor was strengthened by the fact that nervous reactions could develop when the blood already contained neutralizing antibodies against yellow fever virus He cited two illustrative examples one recorded by Sawyer [84] and one from his own experience In this connexion however it may be stated that Macnamara [58] found the sera of four human cases dead of encephalitis following vaccination with French neurotropic virus and revealing on autopsy the presence of the yellow fever vaccine virus in their brain tissues to contain protective antibodies and to be virus free moreover there was no conclusive difference between the neutralizing

indices of the sera of these four encephalitis cases and of sera taken from patients who showed but a mild reaction to the vaccine Further it had been demonstrated by Theiler & Hughes [3] that the sera of rhesus monkeys which had developed a fatal encephalitis following their inoculation with neurotropic virus by extraneural routes invariably contained protective antibodies and that in spite of a high concentration of virus in the animals brains blood taken at the time of death was virus free the virus being confined almost entirely to *nervous tissue*

Durieux (see also page 163) drew attention to the marked disparity between the various results obtained after mass application of the Dakar method Foci of encephalitis had appeared in three territories—French Equatorial Africa (Brazzaville) Costa Rica (La Fortuna Villa Quesada Los Chiles) and Nigeria (Enugu)—where a maximum of 250 000 vaccinations had been performed while in other countries where at least 25 million persons had received their first vaccination against yellow fever cases of post vaccination encephalitis had been of rare occurrence and of scattered distribution Such occurrence had been variously ascribed to the effect of concomitant affections to the action of some latent encephalo myelitogenic virus the pathogenicity of which had been reinforced by the yellow fever vaccination or to the action of the immunizing virus itself even though inoculation of laboratory animals with nervous tissue taken from fatal cases had invariably failed to reveal the presence of the vaccine virus The isolation however by Macnamara [35] of the vaccine virus from the brains of four fatal cases in Nigeria—a finding corroborated at the Institut Pasteur Dakar—confirmed the belief that the use of the French neurotropic virus for human vaccination could in certain cases be considered a direct cause of meningo encephalitis On this basis it was possible to suggest a hypothesis which might explain the occurrence of encephalitic reactions particularly when they appeared in the form of foci confined to a particular area and during a relatively short period of time Experience gained in French West Africa had shown that the general reaction following immunization with Dakar vaccine appeared regularly on the fourth or fifth day Observations made elsewhere during the occurrence of foci of encephalitis however had proved that this general reaction took place only after a certain delay namely from the fifth to the eighth day sometimes indeed the reaction occurred towards the end of the tenth day immediately before symptoms of encephalitis appeared A similar delay had been observed after the use of Laigret s attenuated vaccine (from the 6th to the 11th day) and also after the inoculation of the 17D vaccine which occasioned the outbreaks of encephalitis in Brazil during 1941 when the average period between inoculation and the appearance of the general reaction was from 9 2 to 11 5 days in one area and was 12 3 days in the other Inasmuch as the general reaction occurred during the period of

multiplication of the vaccine virus in the circulatory system a delay in its appearance indicating a slower multiplication of the virus was the result of the inoculation of an inadequate amount of vaccine virus It was considered that the inoculation of a very small quantity of vaccine virus and its slow development in the blood were factors which favoured the occurrence of encephalitis When an adequate amount of vaccine virus was inoculated the virus multiplied rapidly in the blood and antibodies began to form in man on about the seventh post vaccination day it was probable that these antibodies gradually destroyed the circulating virus which had completely disappeared from the blood by the tenth day If on the other hand the amount of vaccine virus inoculated was too small multiplication of the virus took place slowly and the formation of antibodies was retarded in this case it was contended that since the amount of antibody was insufficient to neutralize the virus in the time required the virus persisted in the organism after the tenth day and was then able to penetrate the central nervous system and multiply there On this reasoning therefore the occurrence of encephalitis would in certain cases be the result of the inoculation of very small doses—a state of affairs which could be brought about when the vaccine had been stored under unfavourable conditions prior to use and its potency thereby considerably reduced In Durieux s opinion it was in the use of a vaccine thus modified that the cause of the untoward occurrences reported in certain countries had to be sought—in French Equatorial Africa Costa Rica and Nigeria after the use of Dakar vaccine in Brazil after the use of 17D vaccine in 1941 With respect to the outbreaks in Brazil for example it had been shown that the frequency of encephalitis cases increased with the age of the vaccine would it not be logical to assume that as the potency of the 17D vaccine gradually decreased probably owing to unsatisfactory conditions of preservation the quantity of virus inoculated also gradually decreased and approached the minimal infective dose?

On the hypothesis suggested by Durieux comments were made by Soper and by Macnamara

Soper agreed that an explanation of the Costa Rican cases was to be found in the greatly lowered virus content of the Dakar vaccine at the time of its application in that territory He personally had seen some of the encephalitic reactions there and had also seen classical and fatal cases of yellow fever in persons infected some four or five weeks after vaccination with the same vaccine lot as that used for the vaccination of those who had developed encephalitis This latter observation provided in his opinion important confirmatory evidence of the very low concentration of virus in (even of its absence from) the vaccine applied He refused however to accept the suggestion that low virus content of the 17D vaccine used in Brazil during 1941 could have occasioned the outbreaks of

encephalitis there at that time An increase in the neurotropism of the 17D substrains used in the preparation of the vaccine lots concerned had been proved to be the etiological factor responsible for the outbreaks Moreover in marked contrast with French neurotropic virus there was no laboratory evidence to suggest that 17D virus was more dangerous when administered extraneurally in very small dosage

Macnamara found himself unable to subscribe to the small dose theory of causation The contents of some ampoules from one lot of Dakar vaccine which had been used for most of the vaccinations incriminated in producing the encephalitis cases in Enugu had been shown on later potency titration to be above the minimum standard of the Institut Pasteur Dakar Moreover a follow up survey of Enugu school children who had been vaccinated with this same vaccine lot proved 95% to be immune The untenability of the theory as regards 17D vaccination was evidenced by Macnamara in his reference to the results of a large scale vaccination campaign carried out with 17D vaccine at a town 60 miles (100 km) from Enugu At the end of the campaign less than 50% of the sera collected from those who had been vaccinated were found to have become protective and it was presumed that due either to some factor in storage or to delay in vaccination the virus dosage must have been unduly low yet in that town no cases of encephalitis were reported

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Since this paper was written accounts of further cases of encephalitis following 17D vaccination in recent years have been published

One reported by M Lartigaut and D Lartigaut (*J Med Bordeaux* 1954 **131** 1388) followed the inoculation of a 3 months old infant in France on 12 May 1954 On the eighth post vaccination day the infant became restless and feverish By the 11th post vaccination day the temperature had risen to 38 2° 39 9°C and four or five times a day there were slight convulsive attacks with trismus deviation of the eyes to the right and transient muscle spasms in the upper and lower extremities Clinical examination in the evening of the 11th post vaccination day revealed slight myosis marked rigidity of the neck presence of Kernig s and Brudzinski s signs considerable vasomotor disturbances no other abnormal physical signs no bulging of the anterior fontanelle On lumbar puncture the cerebrospinal fluid was clear and under increased pressure it contained 40 cells per mm³ (lymphocytes 90% polymorphonuclear cells 10%) it had albumen sugar and chloride contents of respectively 60 mg % 70 mg % and 750 mg % it was free from micro organisms On the 12th post vaccination day the temperature remained at 40°C throughout and there occurred another convulsive attack Thereafter defervescence

was rapid and progressive no further convulsive attacks occurred all signs of meningeal irritation disappeared By 27 May one week after the onset of illness the child had completely recovered

Another case reported by E A Beet (*Brit med J* 1955 1 226) occurred in a 3 months old baby boy who while in the United Kingdom in 1954 had been vaccinated against smallpox on 30 October and against yellow fever on 22 November and who had arrived with his parents at Kano Nigeria on 30 November On 3 December he was admitted to hospital as he refused to take his feeds On 4 December i e 12 days after his yellow fever vaccination his rectal temperature was 103 F (39 5°C) he developed a Jacksonian attack involving the left limbs this was accompanied by a left facial palsy and deviation of the eyes to the left No mention is made of treatment or of duration of illness but th child is stated to have done well and to have been restored to norma health

A third case reported by L de Castro Freire (*Rev port Pediat* 1955 18 65) occurred in Portugal and followed the vaccination of a 6 weeks old baby girl on 25 September 1954 The 17D virus vaccine used in this instance had been prepared in Brazil and issued by the Institute of Tropical Medicine Lisbon On 6 October i e on the 11th post vaccination day the child suddenly became feverish and restless Clinical examination two hours after onset of symptoms revealed a rectal temperature of 39 5 C and some redness of the fauces but no abnormal signs in the nervous system abdomen thorax or ears During the night of 6-7 October the child had two convulsions and on the morning of 7 October suffered a third although a less severe convulsive attack On 7 October the con dition was as follows the temperature varied between 40 2 C and 39°C periods of somnolence were succeeded by moments of hyperexcitability there was evidence of meningeal irritation Brudzinski s sign was positive the reflexes were overbrisk ankle clonus was well marked in the right lower extremity less so in the left the cerebrospinal fluid contained albumen 55 mg / sugar 75 mg / and 360 cellular elements per mm³ among which the polymorphonuclear cells predominated the fluid was found to be bacteriologically negative there was no further convulsion Treatment consisted in the administration of Aureomycin and 1 ampoule of gamma globulin October 8 and 9 brought little change in the child s condition treatment was with 2 ampoules of gamma globulin followed by Terramycin and later by quinine suppositories On 9 October the cerebrospinal fluid contained albumen 80 mg / sugar 70 mg / and 332 cellular elements per mm³—elements which included a number of red cells and revealed as on 7 October a preponderance of polymorpho nuclear cells over lymphocytes the fluid was again bacteriologically negative Convalescence began on 10 October the child took her food

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well and her reflexes were less brisk she was however still somewhat excitable if disturbed

During her convalescence which was uneventful and was not complicated by any nervous sequelae she was given injections of vitamins C and B When examined one month later on 8 November she was found to have made a complete recovery The electro encephalographic findings were in every respect normal for a 6 weeks old infant

A fourth case, reported by W O Thomson (*Brit med J* 1955 2 182) followed the inoculation with 17D virus vaccine of a 7 weeks old baby boy on 24 July 1953 at the Immunization Clinic Glasgow Scotland Seventeen days after his inoculation the child became cross and feverish and remained in that state for the next two days he had no bowel or feeding disorders He became increasingly drowsy however and on the 20th post inoculation day his eyes began to roll his limbs started to twitch and he had a frank convulsion on that day his temperature was 102 F (38 9°C) and he was admitted to hospital On admission he was restless and irritable and had a temperature of 100 8°F (38 2°C) but showed apart from exaggerated tendon reflexes no other abnormality shortly after admission he developed three further convulsive seizures Blood examination revealed a leucocyte count of 13 000 per mm³ The cerebro spinal fluid was clear and not under increased pressure contained protein 100 mg % with an increase in globulin sugar 37 2 mg % and chlorides 738 mg % had a cell count of 55 per mm³ (90% lymphocytes 10% poly morphonuclear cells) and showed no acid fast bacilli in film and no growth on culture Two days after admission the temperature returned to normal but further examination of the cerebrospinal fluid on the second fifth and eighth days after admission revealed a raised protein content reaching a maximum of 140 mg % but falling to 30 mg % with an increase in globulin The cell counts remained elevated reaching a maximum of 88 per mm³ but falling to 11 in the final sample with the lymphocytes always in the majority There was at no time any growth on culture and no acid fast bacilli were ever demonstrated The Mantoux test was negative and a radiograph of the chest revealed no abnormality The infant made a rapid recovery without antibiotic therapy and was discharged from hospital 16 days after admission At a follow up examination three weeks later the infant was found to be well without any sequelae

In connexion with this case it has to be noted that two days before the onset of the signs of illness the infant had been vaccinated against smallpox but this was unsuccessful there was no reaction either at the site of the smallpox vaccination or at the site of the inoculation against yellow fever

A fifth case which followed the inoculation on 9 August 1952 of a 5 weeks old baby boy in South Africa has been reported by S Swift (*Brit med J* 1955 2 677) Three weeks after his inoculation the boy who in the meantime had arrived with his parents in London became lethargic and took his feeds drowsily falling asleep in the middle of the feed Examination at the time i e on 30 August revealed an elevation of the temperature and a bulging of the fontanelle In the afternoon of that day the boy was admitted to hospital where a lumbar puncture was performed This showed the cerebrospinal fluid to be under slightly increased pressure and to contain 70 cells per mm³ 75 mg / protein and 64 mg / sugar A diagnosis of meningo encephalitis was made Over the following six days the temperature fell gradually to normal The cerebrospinal fluid one week later was normal except for the presence of 55 mg / protein The boy s convalescence was uneventful and uncomplicated by any nervous sequelae

With respect to this case it is of interest to observe that the infant was vaccinated against smallpox and inoculated with yellow fever vaccine on the same day and that the vaccination was unsuccessful

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INTERNATIONAL REGULATION

INTERNATIONAL REGULATION OF YELLOW FEVER VACCINATION

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Introduction

The fact that yellow fever is no longer the fearful scourge that ravaged parts of the world in the nineteenth century is due to the effective preventive measures which have been and are still taken on the national level by the responsible administrations and on the international level through regulations designed to prevent the spread of the disease throughout the world To this end land sea and air transport is subjected to the various requirements contained in international sanitary regulations The basis however is to be found in one general measure which is compulsory in certain clearly defined cases and the efficacy of which is no longer questioned — yellow fever vaccination

The International Sanitary Regulations (World Health Organization Regulations No 2) which came into force on 1 October 1952 state in Article 72 [9]

1 Vaccination against yellow fever shall be required of any person leaving an infected local area on an international voyage and proceeding to a yellow fever receptive area

2

3 A person in possession of a valid certificate of vaccination against yellow fever shall not be treated as a suspect even if he has come from an infected local area (page 8)

The problem connected with infected local areas with yellow fever receptive areas or endemic zones or with disinsectization of airports and of the various means of transport which are covered by international health legislation are beyond the scope of a monograph on yellow fever vaccination But it may be useful to recall the provisions of the International Sanitary Regulations concerning yellow fever vaccination and to depict the role played in this field by the World Health Organization

It is provided that the certificate of vaccination or revaccination against yellow fever in order to be considered valid in international traffic must conform to the rules laid down and to the model given in the International Sanitary Regulations (see Annex 1 page 204)

These rules are as follows [9]

This certificate is valid only if the va cine used has been approved by the World Health Organization and if the vaccinating centre has been designated by the health administration for the territory in which that centre is situated

The validity of this certificate shall extend for a period of six years beginning ten days after the date of vaccination or in the event of a revaccination within such period of six years from the date of that revaccination (page 48)

Background

When the Fourth World Health Assembly on 25 May 1951 adopted the text of the International Sanitary Regulations it retained with slight modification a provision which appeared in Article 1 paragraph X of the *International Sanitary Convention for Aerial Navigation of 12 April* 1933 as amended by the International Sanitary Convention for Aerial Navigation 1944 The part of this Convention to which reference is made reads as follows

Article 1 [Article III of the Amendment]

X A *valid anti yellow fever inoculation certificate* is one certifying that the bearer has been inoculated against yellow fever with a vaccine and by a method approved by UNRRA

In addition the International Sanitary Convention for Aerial Navigation 1933/1944 specifies

Article 36 [Article XI of the Amendment]

(10) That UNRRA shall lay down standards with which yellow fever vaccine shall conform

(11) That they will make arrangements to test at frequent intervals the activity of the yellow fever immunizing vaccine in use in order to ensure that its immunizing propertie are satisfactory and for this purpose agree that UNRRA in consultation with the governments concerned and as regards the Western Hemisphere with the Pan American Sanitary Bureau *shall designate from time to time* institutes which are approved for the carrying out of such tests

The abo e texts were inserted in the amended International Sanitary Convention for Aerial Navigation 1944 No previous international sanitary conventions or treaties contained any such provisions

The international regulations with regard to yellow fever thus recognized the principle followed in the United States of America whereby persons arriving by air and bearing a recognized vaccination certificate were not subject to any restrictive quarantine measures [3] After the principle had been adopted it was subsequently decided that the certificate of vaccination against yellow fever would be considered valid 10 days after the injection of the vaccine provided that the vaccinated person had continually resided in a non endemic zone if the contrary was the case the certificate was to be valid only after 15 days The duration of validity was four years only [4]

The standards to which yellow fever vaccine was to conform were specified by UNRRA and the rules laid down were based on the methods of manufacture of 17D vaccine perfected in the United States of America and in Brazil by Rockefeller Foundation experts These standards specifying the manner of preparation preservation and administration of the vaccine were published in 1945 in the UNRRA *Epidemiological Information Bulletin* under the title Standards for the Manufacture and Control of Yellow Fever Vaccine They were subsequently adopted by WHO and are still in force The full text of the standards will be found in Annex 2 (see page 205)

For testing the activity of the yellow fever vaccine as provided for in Article 36 of the 1933/1944 International Sanitary Convention for Aerial Navigation UNRRA in consultation with the governments concerned designated a certain number of institutes which were approved for carrying out control tests These institutes were required to decide whether the yellow fever vaccine intended for international use could receive UNRRA s approval

When the WHO Interim Commission took over UNRRA s functions on 1 December 1946 UNRRA had approved the following institutes and laboratories for performing the control tests on yellow fever vaccines

Bogotá (Colombia) Yellow Fever Laboratory National Yellow Fever Service

Dakar (Senegal) Institut Pasteur

Entebbe (Uganda) Yellow Fever Institute

Hamilton (Montana USA) Rocky Mountain Laboratory US Public Health Service

Johannesburg (Union of South Africa) South African Institute for Medical Research

Lagos (Nigeria) Yellow Fever Research Institute

London (England) Wellcome Research Institute

New York (USA) International Health Laboratories Rockefeller Foundation Division

Paris (France) Institut Pasteur

Rio de Janeiro (Brazil) Yellow Fever Research Institute

The following laboratories were also approved for the manufacture of yellow fever vaccines

International Health Division of the Rockefeller Foundation [a]

National Institutes of Health US Public Health Service [a]

South African Institute for Medical Research Johannesburg

Institut Pasteur Dakar [b]

Wellcome Research Institute London

Yellow Fever Laboratory of the Brazilian National Yellow Fever Service Rio de Janeiro [c]

Yellow Fever Laboratory of the Colombian National Yellow Fever Service Bogota

At its fourth session in 1947 [1] *the WHO Interim Commission decided* to have tests of vaccine activity carried out by two or more approved control laboratories on all yellow fever vaccine for which its approval was asked in accordance with the provisions of Article 36 (11) of the 1933/1944 International Sanitary Convention for Aerial Navigation That procedure has been retained by WHO and has been followed since the entry into force of the International Sanitary Regulations on 1 October 1952 on *each occasion on which the Organization s approval has been requested*

Present Procedure for Granting WHO Approval to Yellow Fever Vaccine

WHO considers only applications for its approval transmitted through the health administration of the country in which the institute or laboratory preparing the yellow fever vaccine is situated Applications are to be accompanied by a report giving detailed information with regard to

(*a*) the personnel responsible for the preparation of the vaccine (such personnel must not engage in any work on other viruses or bacteria for the period of the preparation of the vaccine)

(*b*) the premises and material used (which must not be used for any other purpose)

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[a] Fully approved

[b] Approved on condition that the vaccine is administered by the scarification method of the Institut Pasteur Dakar

[c] Provisionally approved for quarantine purposes (full approval was subsequently accorded by WHO)

(c) the preparation technique (inoculation and incubation of eggs harvesting and grinding of embryos filling of ampoules and desiccation must all be carried out in conformity with the approved standards);—

(d) the control tests (which must give satisfactory results)

The activity of the vaccine is then tested This testing is done by three laboratories selected from the list of institutes and laboratories approved for the purpose by WHO

The required number of ampoules (generally six) of a single batch of vaccine packed in dry ice are sent by air to the three control laboratories A copy of the protocol established by the institute manufacturing the vaccine giving the details of the preparation and the results of the titration are sent direct to the World Health Organization but not to the control laboratories which titrate the vaccine independently and communicate the results to WHO

The results of the titrations made by the control laboratories and by the institute which prepared the vaccine are then compared and communicated to certain members of the WHO Expert Advisory Panel on Yellow Fever together with a summary of the report accompanying the application for approval The experts are requested to give an opinion as to whether WHO can accord its approval to the vaccine tested They may call for further information and if they deem it necessary a second control test

The replies of the experts are centralized by the secretary to the Expert Advisory Panel on Yellow Fever and until 1953 they were submitted to the Executive Board of WHO If they were all favourable the Board adopted a resolution approving the yellow fever vaccine prepared by the institute or laboratory for the issue of international vaccination certificates

Since 1954 the Director General has been authorized by the Executive Board subject in each case to the satisfactory completion of the technical procedure now established to grant approval to yellow fever vaccines for the issue of the international certificates of vaccination and revaccination [2]

The decision is communicated to the health administration concerned and published in the WHO *Weekly Epidemiological Record*

Approved Institutes and Laboratories

The institutes at present approved by WHO for the preparation of vaccines for use prior to the issue of international vaccination or revaccination certificates are as follows (see also fig 1)

Australia	Melbourne	The Commonwealth Serum Labo ator es
Brazil	Rio de Ja e ro	Yellow Fever Laboratory Instituto Oswaldo Cruz

FIG 1 INSTITUTES APPROVED BY WHO FOR PRODUCING AND TESTING YELLOW FEVER VACCINES

- Institutes prod ci g WHO app o ed c i es and t sti g the acti ity f acci s
- I stitutes prod ci g WHO appro ed vaccines
- I stit t s t ti g th cti ity of c i

Colombia	Bogotá	Instituto de Estudios Especiales Carlos Finlay
France	Paris	Institut Pasteur
French West Africa	Dakar Senegal	Institut Pasteur de l'Afrique Occidentale Française
Netherlands	Amsterdam	Koninklijk Instituut voor de Tropen Afd Instituut voor Tropische Hygiene en Geographische Pathologie (Institute of Tropical Hygiene and Geographical Pathology)
Union of South Africa	Johannesburg	South African Institute for Medical Research
United Kingdom of Great Britain and Northern Ireland	Beckingham Kent	The Wellcome Research Laboratories
United States of America	Swiftwater Pa	The National Drug Company

The laboratories approved by WHO for testing the activity of yellow fever vaccine are as follows (see also fig 1)

Australia	Melbourne	The Commonwealth Serum Laboratory
Brazil	Rio de Janeiro	Yellow Fever Laboratory Instituto Oswaldo Cruz
Colombia	Bogotá	Instituto de Estudios Especiales Carlos Finlay
France	Paris	Institut Pasteur
French West Africa	Dakar Senegal	Institut Pasteur de l'Afrique Occidentale Française
India	Bombay	Haffkine Institute
Malaya (Federation of)	Kuala Lumpur	Institute for Medical Research
Netherlands	Amsterdam	Koninklijk Instituut voor de Tropen Afd Instituut voor Tropische Hygiene en Geographische Pathologie (Institute of Tropical Hygiene and Geographical Pathology)
Nigeria	Lagos	Virus Research Unit West African Council for Medical Research Laboratories Yaba
Uganda	Entebbe	Virus Research Institute
Union of South Africa	Johannesburg	South African Institute for Medical Research

United Kingdom of Great Britain and Northern Ireland	Beckingham Kent	The Wellcome Research Laboratories
United States of America	Bethesda Md	Laboratory of Biologics Control National Institutes of Health

The International Sanitary Regulations contain no provision covering the yellow fever immunity certificate mentioned in the 1933/1944 International Sanitary Convention for Aerial Navigation WHO therefore no longer keeps a list of laboratories and institutes approved for the issuance of such certificates

Vaccination Centres

A list of all the centres authorized by health administrations to issue international yellow fever vaccination or revaccination certificates is published once a year by WHO The most recent of these lists appeared in a supplement to the *Weekly Epidemiological Record* of 11 February 1955 [8] and contains the names of 1 003 vaccination centres distributed as follows

Europe	140
Asia	57
Africa	543
Americas	228 [d]
Oceania	35

WHO does not participate in the designation by health administrations of centres for vaccination against yellow fever it confines itself to receiving notification of such designations and to transmitting the information to other health authorities by means of the *Weekly Epidemiological Record* Nevertheless the Organization may request particulars as to the origin of the vaccine employed and assure itself that the centre is provided with facilities for refrigeration at between 0° and 4°C in order to guarantee proper storage of the vaccine

These centres are naturally entitled to issue yellow fever vaccination or revaccination certificates in accordance with the model shown in the International Sanitary Regulations (see Annex 1)

Vaccination Certificates

A number of details must be indicated on the international certificate of vaccination or revaccination against yellow fever the vaccinated person s name date of birth sex and signature The vaccinator must insert

[d] Not including centres in Mexico

the date in the following order day month year the month being indicated in letters and not by a figure In the appropriate sections he must note the origin of the vaccine employed and the batch number the certificate must be signed in his own handwriting and stamped with the centre s official stamp

Every effort must be made to avoid corrections erasures or omissions as they might affect the validity of the certificate

It will be seen from Annex 1 that the certificate is printed in English and French The addition of a third language is however authorized provided that it is one of the official languages of the territory issuing the certificate The certificate must be completed in either English or French the addition of another language being optional

The certificate is valid for six years beginning 10 days after the date of vaccination or in the event of revaccination within such period of six years from the date of that revaccination It has however often been proved that effective immunity commences from the seventh day after vaccination and that it persists for several years beyond the six year limit recommended (see page 105)

The Regulations make no provision for contra indications to vaccination on medical grounds or for exemption from vaccination under a certain minimum age Nevertheless if a vaccinator considers that vaccination is contra indicated on medical grounds he issues to the person concerned a certificate stating the medical facts he has observed and his reasons for having considered vaccination undesirable The health authorities in the place of arrival may accept such a certificate but remain entirely free to adopt quarantine measures with respect to a person holding it

With regard to yellow fever vaccination of infants under one year old the WHO Committee on International Quarantine expressed the opinion that each country must make its own decision on this point weighing the risk of importing yellow fever through non vaccinated infants against the risk such infants run in undergoing vaccination The Committee recommended that in any case the dose of vaccine employed should be the same for infants as for adults and that in no circumstances should it be reduced [4]

The practical considerations concerning yellow fever vaccination thus create certain problems for WHO Those concerning the approval of yellow fever vaccines are even more complex

—

[4] o Ceylon I d a d Pak stan a r servat on appl e wh reby in the case of a perso v cc ated in a yellow fe r e dem c zo e or of a person who h entered ch zo with n 10 days of ac at on the [pre crib d] period of 10 days shall be e tended to 1 d ys

Problems Connected with the Control of Yellow Fever Vaccine

There is no doubt that the provision in the International Sanitary Regulations whereby WHO is made responsible for approval of yellow fever vaccine is a wise one If there were no such approval travell rs would be at the mercy of health administrations who might or might not admit the validity of a certificate and might insist on the use of a particular vaccine Moreover all countries and in particular those which are receptive to yellow fever are entitled to benefit from the protective measures for which the Regulations provide

WHO therefore being responsible for the application of the International Sanitary Regulations is obliged to provide itself with every possible guarantee before approving yellow fever vaccine It is also evident that once a vaccine has been approved by WHO it must maintain the qualities it had when it was tested before receiving official recognition This is one of WHO s problems but there are several others which await solution

For example tests to ascertain that there is no bacterial or virus contamination and to identify the virus as well as innocuity tests are naturally made by the institute preparing the vaccine but would it not be desirable to have such tests made by other institutes as well ?

In the course of time there may be changes in the personnel premises equipment and methods used Should WHO not be informed of any such changes ? Should it not ensure that the quality of the vaccine remains satisfactory and in conformity with the required standards ?

As was seen earlier the standards for the manufacture and control of yellow fever vaccine were established by UNRRA in 1945 These standards are still in force in their original text Are the minimum of 150 000 LD_{50} (mouse units) of 17D virus per ml of vaccine and the 500 LD_{50} which each dose of virus injected must contain—as prescribed by these standards—still satisfactory ?

Is it not time to take account of the progress which has been made in culture techniques methods of desiccation and storage of live vaccines ? Yellow fever vaccines administered by scarification are now in use on a large scale but the UNRRA standards make no mention of such methods

The methods employed by the control laboratories for testing the potency of yellow fever vaccine also vary considerably from one laboratory to another To take one example only the diluent used by the various yellow fever laboratories in their virus titration techniques may be depending on the laboratory bovine albumin (Cohn fraction V) human serum

or horse rhesus monkey or rabbit serum it may be suspended in distilled water or in $8^0/_{00}$ $8.5^0/_{00}$ or $9^0/_{00}$ physiological saline the serum us d may be from a single subject or from a serum pool It is inevitable that such diverse techniques give results that are not always strictly comparable

All these questions are b ing studied under the auspices of WHO The Expert Committee on Yellow Fever at its second session in September 1953 at Kampala Uganda made recommendations to this end [3] Surveys and investigation are in progress and although it is as yet too early to give the results they demonstrate the fact that WHO is well aware of its responsibilities in the field of yellow fever vaccination

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Annex 1

INTERNATIONAL CERTIFICATE OF VACCINATION OR REVACCINATION AGAINST YELLOW FEVER

CERTIFICAT INTERNATIONAL DE VACCINATION OU DE REVACCINATION CONTRE LA FIÈVRE JAUNE

This is to certify that / Je soussigné(e) certifie que } date of birth / né(e) le } sex / sexe } —

whose signature follows / dont la s gnature suit }

has on the date nd cated been vaccinated or revacc nated against yellow fever
a été vacciné(e) ou revacciné(e) contre la fièvre jaune à la date indiquee

Dat	S gn re and professional ta us of accinator S gna re et quali é professionnelle d accinate	O gi and b ich of vaccine Origine d accin empl yé n méro du lot	Official st mp of vaccinati g centre Cachet fficiel d centre de accinatio	
1			1	2
2				
3			3	4
4				

Th cert fica is valid onl f he vaccine used has been pproved by he W rld H lth Org ni tio and f the centre ce re has bee des g ted by h heal h admini ration f the ter tory in hich ha cen re is si uated.

The va di y of hi cert fic hall e end f period of si years, beg ni g d ys fter he date of accination or in th ve f acc wi h h pe od f ye rs, from d te of that revaccination.

A amendment of thi certificate, or erasure or fail re to comple an part of eu render it invalid.

Ce cert ficat es al ble que si le accin em loyé été pprouvé pa l'Organisation Mond ale de l Santé et si le cen re d ccin été h b l é par l dmini ration sanitaire d territoire dans lequel ce centre est ué.

La alidité de ce cert ficat couvre ne période de ns commençan dix jours près la date de la accination ou, dans l cas d ne re accin i cours d ce te pé ode de ix ans, le jour de cette revaccination.

Toute correctio ou rature sur le certificat ou l omissio d'une quelconque des mentions qu'il comporte peut affecter sa l di é.

ANNEX 2

STANDARDS FOR THE MANUFACTURE AND CONTROL OF YELLOW FEVER VACCINE

Min mum Requirements

1 *Type of acci e*

The vaccine shall be of the aqueous base (serum free) type The production method employed shall be essentially as describ d in the reports referred to below [1] A rigid aseptic technique must be employed during all stages of production The finished virus suspension contains only virus infected ch ck-embryo pulp suspended in distilled water and must be free of all other viable micro organisms as determined by suitable tests The finished vaccine shall be dried in the final container from the frozen state under high vacuum by an acceptable method The dried product shall contain not more than 1 0° of moisture (preferably not more than 0 5) as determ ned by the phosphorous-pentoxide—vacuum method The finished vaccine shall be flame sealed in an ampoule of su table size and prepared from glass of not less than USP type I quality Prior to sealing the ampoule shall be filled to atmospheric pressure with pure moisture free n trogen

2 *I lentity and p ty f the iru stra*

The acceptable primary seed virus is known as the 17D strain of yellow fever virus and shall be of the 00th 300th subculture [2] It shall be satisfactory with regard to the following characteristics in the monkey (these are discus ed in detail in references 3 and 4)

(*a*) The degree of viscerotrop sm as indicated by the amount of circulat ng irus following intracerebral inoculation

(*b*) The onset and duration of a typical febrile reaction

(*c*) The degree of neurotropism as indicated by the i cidence of clinical man festa tion of encephal tis and death

(*d*) The development of an immune response as measured by the abil ty of the serum to prevent infection in the mouse

The virus must be f ee of all other viable micro-organisms as determ ned by suitable tests The seed lot system [3] shall be employed in preparing and ma ntaining the stock of secondary seed virus

3 *M nkey safety test o the seco da y seed i u*

The object of this test is to evaluate the behaviour characteristi s of the virus in a susceptible animal as determined by tests on the secondary seed irus using ot less than 6 rhesus monkeys The test dose is injected intracerebrally i to healthy rhesus monkeys (*Macaca m latt*) which have proved to be non immune to yellow fever by virus neutral zation tests of blood specimens drawn from each monkey just prior to injecting the seed irus The monkeys s bsequently shall be observed for a minimum period of 30 days Not more than one (16) of the anim ls shall fa l to become immune

Rep d ed w th cert n e1tor l mod fcat ons f om the *Ep d m l e I I f mat n B ll t* 1945 1 365

to yellow fever as determined by virus neutralization tests of blood (serum) drawn 14 or more days subsequent to inoculation of the vaccine Not more than 2 (33 %) of the monkeys under test shall develop encephalitis which may be manifested by paralysis or inability to stand and which may or may not result in the death of the animal Blood serum obtained from each of these test monkeys on the 2nd 4th and 6th day when injected in tenfold dilutions intracerebrally into suitable mice shall cause deaths in lower dilutions but not more than LD_{50} death rate in dilution 10^{-2} due to a specific yellow fever virus encephalitis during an observation period of 21 days Not less than 6 mice shall be used at each dilution Each lot of seed virus must pass satisfactory monkey safety and immunity tests [4,5]

4 *Chicken embryos*

Only living typical chick embryos from virus inoculated eggs shall be harvested The age of the embryo shall be computed from the hour of initial introduction into the incubator Although the age of the harvested embryo is not inflexibly defined it preferably should be not more than 11 days [1] The inclusion of the heads of the embryos in preparing the pulp is optional

5 *Potency of the finished vaccine*

The finished dried chick embryo pulp when rehydrated to its original volume shall contain not less than 150 000 MLD of 17D virus per millilitre of vaccine at the time of passing final potency test Present information indicates that for the satisfactory immunization of man not less than 500 MLD of active virus should be contained in the immunizing dose injected an excess being preferred

(One MLD [LD_{50}] is defined as that quantity of yellow fever virus which when inoculated intracerebrally into each of an adequate number of yellow fever susceptible adult mice (16-20 g) will kill 50% of the animals due to a specific yellow fever virus encephalitis within 21 days See reference 6 for method of determining 50% end point)

6 *Guinea pig safety tests*

A safety test in the guinea pig shall be made on each lot of finished vaccine using rehydrated chick embryo pulp from ampoules selected at random from the filling Four to 5 ml shall be injected intraperitoneally into each of 2 or more normal guinea pigs weighing 300 g to 500 g The animals shall be observed for 7 or more days during which time no significant clinical manifestations shall occur If both of the animals show significant reactions the entire lot shall be regarded as unsatisfactory If one animal shows significant clinical reaction the test shall be repeated using 3 test animals If in the repeated test more than one of the three animals show significant reactions the product is unsatisfactory

7 *Sterility of the vaccine*

Sterility of the product must be maintained at all times while processing As a final check the finished vaccine shall be sterile as indicated by tests on not less than three final containers selected at random when the total filled is 100 or less plus one additional container for each additional 50 containers filled Not less than 1 ml from each container or the entire contents if less than 1 ml of the chick-embryo pulp when rehydrated as directed on the label shall be tested Duplicate cultures shall be made with incubation at 37 C and 22 C If contamination appears in any of the tubes planted the test may be repeated with the same number of containers and a lot shall be discarded

if the same type of organism appears in more than one test but no lot shall be passed until the final test shows no growth throughout

8 Ide tity test

The potency test on the finished vaccine shall be considered a satisfactory identity test

9 Lab lling

The outside label on each container shall show

(*a*) the official name of the product namely Yellow Fever Vaccine

(*b*) *the statement Li ing dried culture of 17D virus prepared from infected chicken* embryo

(*c*) the volume and kind of diluent to add

(*d*) dose

(*e*) the lot number and expirat on date

(*f*) the licence number name and address of manufacturer

(*g*) a statement of the conditions of storage (see section 10)

(*h*) a statement of the volume of chick embryo pulp before drying is optional

( ) the statement No U S Standard of Potency shall *t* appear

10 Expirati n date

The dating period of yellow fe er va cine is divided into three per ods

(*a*) Period of very low temperature storage between harvest ng and the time of passing a satisfactory potency test (the latter date is designated the date of manu facture) This is a variable period dependent upon the manufacturing conditions demands for vaccine and storage temperature

(*b*) Period between date of manufacture and date of beg nning distribution (the latter is designated the date of issue) This per od shall be not more than 1 year after date of ma ufacture and during th s time the product must be kept constantly at a temperature below –5 C (23 F) preferably below – 0 C

(*c*) Period of distribution This period (ending with the exp ration date) shall be not more than 1 year after the date of issue and during this time the product must be kept constantly at a temperature below 5°C (41 F) and the label must bear the st te ment followi g the e p ation date If kept below 5 C (41 F) Keep preferably below 0°C (3 F)

11 Adm st at on of the acci e

Yellow fever vaccine processed in accordance with these min mum requ rements is prepared for human injection in the following manner draw into a sterile needle and syr nge of suitable size the diluent (sterile physiological sodium chloride solution) on the basis of 10 ml of d luent for each 1 ml of dried ccine as shown on label of ampoule then open the vaccine conta i g ampoule and carefully mix the vacc ne and the d luent unt l all has been drawn into the syr nge and is in a un form suspens on Because of the volume n case of the larger ampoules the l quefy ng and dilut g w ll nee l to be accompl shed w th a separate bottle of d luent and a suitable syringe Administer the *single imm n i g d* of 0 5 ml subcutaneou ly *at e* Children recei e the same dose as adults

12 Shipping requirements

Yellow fever vaccine shall be shipped in a suitable container adequately packed in carbon dioxide ice or provided with other means of refrigeration so as to ensure a temperature constantly below 0 C

13 General requirements

All other requirements as to methods of manufacture and the maintenance of adequate processing and distribution records which are required by governmental regulations shall apply

14 Requirements for release

Where required by governmental regulations as in the United States distribution of each lot shall be withheld pending official release For this release there shall be submitted the following

(*a*) A protocol of the potency and sterility tests

(*b*) No less than 3 vial or not less than the equivalent of 50 cm³ of finished vaccine shipped as directed in section 12

DIRECTIONS FOR PRESERVATION OF SEED VIRUS

The primary seed virus shall be preserved in the dried state under nitrogen and stored continuously at −70 C or colder The virus containing material is distributed into ampoules of USP type 1 glass or better shell frozen quickly following which the material is dried from the frozen state under high vacuum until the moisture content of the virus containing material is less than 0 5 % The ampoules are then filled with dry nitrogen flame sealed inspected labelled and immediately stored at −70 C or colder If properly prepared and stored the virus will remain viable for an indefinite number of years

The secondary seed virus is prepared as chick-embryo pulp by the method described for the preparation of the pulp for vaccine production The pulp is ampouled dried and stored in the manner described for the preparation of the primary seed virus

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